Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

.9 84 M
\lsc. Publ. 1005
. 1

Proceedings:

CONFERENCE ON ESTROUS CYCLE CONTROL IN DOMESTIC ANIMALS

July 9-10, 1964 Nebraska Center
for Continuing Education
University of Nebraska
Lincoln, Nebraska

BELTSVILLE BRANCH
AGRICULTURE
1965
LIBRARY

COOPERATIVE STATE RESEARCH SERVICE AND AGRICULTURAL RESEARCH SERVICE
U. S. DEPARTMENT OF AGRICULTURE IN COOPERATION WITH UNIVERSITY OF NEBRASKA

MISCELLANEOUS PUBLICATION 1005

CONFERENCE PROGRAM

THURSDAY, JULY 9

A.M.

8:30	Registration
9:00 - 9:15	Welcome to Conference Dean E. F. Frolik University of Nebraska
	Objectives C. F. Sierk U.S. Department of Agriculture
9:15 - 10:30	Evaluation of Methods for Controlling the Estrous Cycle Wm. Hansel, Chairman R. G. Zimbelman J. F. Wagner
10:30 - 10:45	Recess
10:45 - 12:00	Discussion
12:00 - 1:00	Lunch

P.M.

1:00 - 2:00	Some Side-Effects and After-Effects of Experimental Endocrine Treatments Used to Modify the Natural Estrual Rhythm L. E. Casida, Chairman P. J. Dziuk
2:00 - 3:00	Discussion
3:00 - 3:15	Recess
3:15 - 4:15	Mechanisms Controlling the Formation and Persistence of the Corpus Luteum R. M. Melampy, Chairman L. L. Anderson B. N. Day H. G. Spies
4:15 - 5:00	Discussion
6:30	Dinner

FRIDAY, JULY 10

A.M.

8:30 - 9:30	Roles of Hypothalamus and Hypophysis in the Control of the Estrous Cycle A. V. Nalbandov, Chairman M. T. Clegg
9:30 - 9:45	Recess
9:45 - 10:45	Discussion
10:45 - 12:00	Unfinished Business
12:00 - 1:00	Lunch

P.M.

1:00 - 3:00	Summary and Conclusions H. H. Cole

FOREWORD

This publication is a record of the papers presented at a conference on estrous cycle control in domestic animals, held July 10-14, 1964 at the University of Nebraska. Objectives of the conference were to review the status quo of research on this problem, to provide a forum for exchange of ideas and experiences, and to point up the direction of future research.

Research on controlling or synchronizing the estrous cycle in domestic animals has been in progress since Hans Selye at McGill University in 1936 and William Phillips at the University of Rochester in 1937 demonstrated that injected progesterone inhibited ovulation in the rat. R. H. Dutt and L. E. Casida at the Wisconsin station in 1948 successfully controlled estrous and ovulation in ewes. Subsequent experiments with swine and cattle showed varying effects of progesterone injections.

In 1959 oral administration of progestationally active compounds resulted in inhibition of estrous and ovulation in rats. Since that time considerable effort by research personnel at land-grant universities, USDA laboratories and pharmaceutical firms has been devoted to developing and perfecting a practical method for control of the estrous cycle in domestic animals. Concurrently, there has been substantial progress in attempts to elucidate the mechanisms through which progesterone exerts its inhibitory influence on estrus as well as the roles of the hypothalamus and hypophysis in the control of the estrous cycle.

Correspondence and discussions with a number of research personnel and administrators resulted in practically unanimous agreement that a conference on the rather specific topic of estrous cycle control in domestic animals would contribute to a more coordinated research effort and a better understanding of the needs for additional research.

The 12 invited speakers and their papers drew an attendance of 100 from 35 States and 2 foreign countries. Among those attending were representatives from the fields of animal and veterinary science at State, Federal, and commercial research laboratories.

The assistance of Dr. B.N. Day and Dr. Dwane Zimmerman in planning the program and of Dr. William Hansel, Dr. L. E. Casida, Dr. R. M. Melampy, Dr. A. V. Nalbandov and Dr. H. H. Cole in conducting the conference is gratefully acknowledged.

For their willingness to host the conference and their assistance with local arrangements, thanks are due the University of Nebraska and its Center for Continuing Education, whose representatives helped so agreeably throughout the planning and execution of the conference.

C. F. Sierk
Conference Chairman
Cooperative State Research Service
U.S. Department of Agriculture

July 1965

CONTENTS

Page

Evaluation of Methods for Controlling the Estrous Cycle . . . William Hansel . . . 1

Evaluation of Some Methods for Controlling the Bovine Estrous Cycle . . . R. G. Zimbelman . . . 18

Hormonal Control of Reproductive Activity in the Ewe . . . J. F. Wagner . . . 29

Discussion . . . 39

Some Side-Effects and After-Effects of Experimental Endocrine Treatments Used to Modify the Natural Estrual Rhythm. . . L. E. Casida . . . 45

Response of Sheep and Swine to Treatments for Control of Ovulation . . . P. J. Dziuk . . . 50

Discussion . . . 58

Mechanisms Controlling the Formation and Persistence of the Corpus Luteum . . . L. L. Anderson, R. M. Melampy . . . 64

The Lifespan of Corpora Lutea Induced During the Luteal Phase of the Estrous Cycle in Swine . . . B. N. Day . . . 78

Factors Influencing the Regression of Corpora Lutea in the Ewe, Rabbit and Rat . . . H. G. Spies . . . 83

Discussion . . . 86

Roles of Hypothalamus and Hypophysis in the Control of the Estrous Cycle . . . A. V. Nalbandov . . . 92

The Role of Light in the Regulation of Cyclical Estrous Activity in Sheep . . . M. T. Clegg, H. H. Cole, W. F. Ganong . . . 96

Discussion . . . 104

Comments and Conclusions Concerning Studies on Estrous Control in Domestic Animals . . . H. H. Cole . . . 115

PROGRAM PARTICIPANTS

E. F. Frolik	University of Nebraska
L. L. Anderson	Iowa State University
L. E. Casida	University of Wisconsin
M. T. Clegg	University of California
H. H. Cole	University of California
B. N. Day	University of Missouri
P. J. Dziuk	University of Illinois
Wm. Hansel	Cornell University
R. E. Hodgson	Agriculture Research Service, U.S. Department of Agriculture
R. M. Melampy	Iowa State University
A. V. Nalbandov	University of Illinois
C. F. Sierk	Cooperative State Research Service, U.S. Department of Agriculture
H. G. Spies	Kansas State University
J. F. Wagner	Eli Lilly and Company
R. G. Zimbelman	Upjohn Company
D. R. Zimmerman	University of Nebraska

PROCEEDINGS: CONFERENCE ON ESTROUS CYCLE CONTROL IN DOMESTIC ANIMALS

University of Nebraska, July 9-10, 1964

EVALUATION OF METHODS FOR CONTROLLING THE ESTROUS CYCLE

William Hansel
Cornell University, Ithaca, New York

Introduction

Truly effective estrous cycle synchronization techniques are of great potential value to commercial producers of beef and dairy cattle, sheep, and swine. Many commercial beef cattlemen recognize the merits of using outstanding sires through artificial insemination (AI) to produce greater numbers of faster gaining cattle having the most desirable carcass characteristics. The relatively high heritability of feedlot gains makes this idea particularly attractive. Nevertheless, progress in attaining this goal has been rather slow, mainly because of the practical problems involved in breeding range cattle artificially and the low conception rates frequently obtained. Methods for cycle regulation compatible with the most commonly used management practices can play a major role in facilitating the widespread use of artificial insemination and improved selection techniques in the beef industry.

The major application of estrous cycle regulation techniques in dairy cattle is in heifers rather than lactating cows. A large percentage of dairy heifers are presently bred naturally while on pasture to bulls of questionable or unknown genetic merit. This is a dubious practice when one considers that the average dairy cow in the major dairy States, such as New York, produces an average of only 3.5 calves in her lifetime. If heifers can be grouped and their cycles synchronized more of them are likely to be bred artificially to proven bulls.

Cycle synchronization of sheep and swine would result in more uniform lamb and pig crops and better use could be made of facilities and labor. Lambings and farrowings could be concentrated in shorter periods of time, and in the case of swine, the operation of multiple farrowing programs would be facilitated.

Estrous cycle regulation should be particularly useful in underdeveloped areas of the world where transportation and refrigeration facilities are limited and management practices are such that the animals are not frequently observed.

Finally estrous cycle regulation methods provide potentially useful new experimental tools. They can be used to improve the precision and simplify the operational details of many experiments in reproductive physiology and nutrition. They possibly may provide impetus for a large number of new experiments on ovum collection, storage, and transfer in domestic animals.

Since Anderson et al., (1)[1] recently presented a thorough review of the pharmacological control of estrous cycles and ovarian functions, it is felt that the present discussion might most profitably be confined to the period since about 1960 when the first experiments on estrous cycle regulation by feeding steroid hormones to ruminants were conducted. Numerous experiments conducted prior to that time showed, in general, that effective cycle synchronization could be obtained in cattle, swine, and sheep (cycling and anestrous) by various regimens of progesterone, estrogen, and gonadotropin injections. However, the conception rates reported for animals bred at the synchronized estrus were usually quite low, and none of the methods tried appeared readily adaptable to widespread use under field conditions.

In July 1959, at a conference on Physiological Mechanisms Concerned With Conception held at West Point, N.Y. (Hartman, 10), Pincus presented the results of the studies he and his associates had carried out on the use of various orally active steroids as ovulation inhibitors in rabbits, mice, and humans. The progesterone derivatives were most active in ovulation inhibition, particularly the 17 substituted ones including the 6-alpha methyl derivative of 17-acetoxyprogesterone. The possibility that some of these compounds might be useful as estrous cycle regulators in farm animals was apparent and reports concerning their use in both nonruminants and ruminants soon appeared. Nellor (12) reported the successful control of the time of estrus and ovulation in gilts by feeding 6-methyl-17-acetoxyprogesterone, and Hansel et al., (7) reported successful synchronization of cycles in cattle as a result of feeding the same compound. Similar reports concerning the use of this compound in sheep were subsequently published (Hogue et al., 11; Evans et al., 3, and Southcott et al., 14).

---

[1] Underscored numbers in parentheses refer to Literature Cited section.

Experimental Methods

This report presents a compilation of the results of all estrous cycle synchronization trials with cattle, sheep, and swine conducted at Cornell since January 1960. Results of many of these trials have been published previously. The trials with Holstein heifers summarized in table 1 have been described elsewhere (Hansel, 7, Hansel et al., 8, and Van Blake et al., 15).

The trials with beef cattle (table 2) have been previously described (Hansel et al., 7, 8) with the exception of Experiments 5 and 6. In Experiment 5, 100 crossbred beef cattle located at Briarcliff Farms, Pine Plains, N.Y., were assigned to each of the following groups:

Group 1 - Fed 6-methyl-17-acetoxyprogesterone (MAP) at a level of 240 mg. per head per day for 18 days. Group 2 - Fed 6-chloro- Δ^6-dehydro-17-acetoxyprogesterone (CAP) at a level of 10 mg. per head per day for 19 days. Group 3 - Fed CAP at a level of 10 mg. per head per day and thyro-protein at a level of 9 gm. per head per day for 19 days. Group 4 - Untreated controls.

The crystalline MAP was dissolved in 150 ml. ethanol and added directly to a liquid molasses-urea ration and mixed thoroughly. The CAP, which was supplied as a premix concentrate (1 gm. per lb. of alfalfa meal), was shaken with 1 L of ethanol in a large flask and the entire contents of the flask were added to the liquid ration during mixing. The thyroprotein was also added directly to the liquid ration. The cattle were fed once daily; the ration was hauled in a tank to the feeding lots each morning immediately after the hormones were added and mixing completed. The amount of the ration fed was so regulated that each lot consumed all of the ration offered each day. All cattle had free access to ground corncobs as a source of roughage during the feeding period. The hormone feeding period for the MAP-fed cattle started June 28, 1963, and one day later for the other groups. All cows had calved 50 days or more prior to the beginning of the feeding period. The calves from groups 1, 2, and 3 were removed from the cows 4 days prior to the beginning of the feeding period. All cows were palpated per rectum prior to being placed in each group, and all pregnant cows and those with obvious pyometria were discarded. A few cows in early pregnancy (30 days or less) escaped detection; none of these came in estrus after treatment and they were excluded from the results.

Approximately half of the synchronized cattle from each group were bred artificially and half naturally. The semen used was collected from seven bulls on the farm and was diluted with CUE (Foote et al., 4) extender to approximately 15 million motile spermatozoa per ml. of extender. Standard AI procedures were followed. Inseminations were performed by three different persons.

The natural matings were limited to a single service, after which the cows were removed to a different lot. With a few exceptions, none of the bulls used naturally bred more than two cows on any one day.

The control cows were bred in a similar way during a period lasting from the 7th day of the hormone feeding period to the 10th day after the end of the feeding period. Some unavoidable delay occurred in removing the calves from this group of cows, so that breeding was started 10 days before the calves were removed.

All cows returning to estrus 17-25 days after the synchronized estrus were rebred, either artificially or naturally. Some cows that did not show estrus during the synchronization period came in estrus approximately one cycle later and were bred at that time.

All cows were checked for pregnancy by rectal palpations approximately 60 days after the initial breedings and 35-40 days after the second breedings.

The mature Hereford cows in Experiment 6 were each fed 10 mg. of CAP in approximately 5 lb. of grain for 14 days followed by 6 mg. per day for 4 days. These cows calved 20-86 days prior to the beginning of the trial and the calves remained with them during the entire experiment. Eighteen of the 29 cows had been seen in estrus after calving or had a palpable corpus luteum at the beginning of the trial. The remainder had smooth ovaries and may not have been in estrus after calving. All cows in this experiment were bred artificially as previously described.

The details of Experiments 1-6 (see table 3) conducted with sheep have been discussed elsewhere (Hogue et al., 11 and Brunner et al., 2). The procedures used in Experiments 7 and 8 were identical to those used in Experiment 6 and described by Brunner et al., (2). The exact treatments used in Experiments 7 and 8 are shown in table 3. Lactating ewes were used in Experiment 8. Both CAP and MAP were fed to cycling ewes in Experiment 9. The levels fed are shown in table 3.

The results of experiments with swine outlined in table 4 have not previously been reported. Experiment 1 was conducted to provide preliminary data on the effectiveness of another progestational derivative[2] in inhibiting estrus in gilts and to observe the subsequent fertility of these gilts. Thirty-three Yorkshire and Yorkshire x Berkshire gilts were divided into 3 groups of 11 each on the basis of inheritance and weight. Group 1 served as untreated controls; gilts in group 2 were fed the progestin at the rate of 0.3 mg. per lb. of body wt. per day and those in group 3 at the rate of 0.5 mg. per lb. per day. Groups 2 and 3 were fed the hormone in an amount of a typical swine gestation ration equivalent to 3 lb. per animal per day in equal a.m. and p.m. portions. All gilts were checked daily for estrus and were inseminated artificially at the first estrus following treatment with 50 ml. of semen extended with CUE (Foote et al., 4) to contain 3 billion motile sperm. Five gilts from each treated group and four control gilts were slaughtered 2-4 days after breeding. The number of new corpora lutea were counted and ova were recovered to determine if fertilization had occurred. The remaining gilts in each lot were observed for signs of estrus and rebred if estrus occurred.

Experiments 2 and 3 were conducted to test the effects of sequential estrogen and progesterone treatments on cycle synchronization and fertility in gilts. In Experiment 2, 22 Yorkshire gilts were divided according to weight and ancestry into a treatment and a control group. The treatment group received 16 mg. of 3-methyl ether of ethynyl estradiol (MEE) in the feed daily for 9 days followed by 0.6 mg. of MAP per lb. of body wt. for 9 days. The hormones were administered in an amount of a typical swine gestation ration equivalent to 4 lb. per animal per day, fed in equal a.m. and p.m. portions. All animals were checked daily for estrus and were bred naturally at the first day of estrus. Gilts that did not conceive on first service were rebred at the second post-treatment estrus. All gilts were allowed to complete the gestation period and the numbers of pigs farrowed were recorded.

Experiment 3 was conducted in 1964 in exactly the same way as Experiment 2. Twenty Yorkshire gilts comprised the treatment group and 10 served as controls.

Results and Discussion

The results of the trials conducted with dairy heifers and beef cattle summarized in tables 1 and 2 clearly show that both CAP and MAP suppress estrus and ovulation during the feeding period and that both effectively synchronize the estrous cycles of treated cattle after withdrawal. In general MAP has produced slightly better synchronization than CAP; more of the MAP-treated animals have been in estrus on days 3 and 4 and fewer on days 5 and 6 after hormone withdrawal. Perhaps this result might be expected since CAP is so much more potent than MAP in terms of the dose required to inhibit estrus and ovulation. The minimum effective dose of CAP appears to be approximately 10 mg. per animal per day for animals weighing up to 1,000 lb.; the comparable dose for MAP is approximately 200 mg. per animal per day.

Eighteen-day feeding periods appear to be satisfactory; in the one trial where the feeding period was reduced to 14 days (Experiment 5, table 1) both synchronization and fertility seemed to suffer. However, it is possible to reduce the feeding period to 10 days and obtain satisfactory synchronization if some additional treatment is included to prevent those animals in the group that have recently ovulated from forming a functional corpus luteum. This was accomplished in Holstein heifers (Experiment 4, table 1) by placing self-retaining catheters in the uterus of heifers that had recently ovulated at the beginning of the feeding period. The same effect might be achieved by injecting oxytocin during the "critical" period of days 2-6 of the cycle (Hansel and Wagner, 9).

Individual feeding of the hormones does not appear to be necessary; the group-fed animals performed about as well as those fed individually. Adequate feeder space was provided in all cases. There are no data available allowing for direct comparisons of the effects of various rations on the degree of synchronization attained, but one observation may be pertinent. The Hereford heifers in Experiment 3 (table 2) were fed MAP at a level of 0.4 mg. per lb. of body wt. for 19 days, after which the dose was lowered to 0.05 mg. per lb. of body wt. for an additional 5 days. It was expected that these heifers would come in estrus while being fed the lowered dose as was the case with Holstein heifers (Experiment 4, table 1). Surprisingly, 13 of the 19 heifers that came in estrus did so only after the low dose of MAP was withdrawn. It seems unlikely that the low dose of MAP alone was sufficient to inhibit estrus in these heifers. Unlike the animals in other experiments, these heifers were given free access to fresh-cut green forage (mixed legumes and grasses), and the possibility that this forage contained some antiestrogen or antigonadotropin that delayed the occurrence of estrus cannot be ignored.

The conception rates of the Holstein heifers bred at synchronized cycles ranged from 48 to 70 percent and were lower than the controls in all trials except one. These rates may be acceptable in practice, since the relatively high conception rates obtained at the second post-treatment estrus have usually resulted in approximately 90 percent of the heifers being pregnant after the two breedings. The heifers returning to estrus after the first service remained reasonably well synchronized. It is perhaps noteworthy that the highest conception rate was obtained in CAP-treated heifers (Experiment 2, table 1) that were late in coming into estrus (days 6-9) after hormone withdrawal.

---

[2] SC 10363 - G. D. Searle Company. (Trade names are used in this publication solely for the purpose of providing specific information. Mention of trade names does not constitute guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement of other products not mentioned.)

The fertility of the beef cattle bred after synchronization was considerably lower than in Holstein heifers. However, the fertility of control groups of beef cattle bred artificially has also been rather low. The large-scale trial (Experiment 5, table 2) is of particular interest in this respect since it provides the only direct comparisons available among controls and CAP and MAP fed cattle.

Fourteen of the 400 cattle allotted to the four groups in this trial were lost to the experiment as a result of losing their identification or escaping from their assigned lots. Effective synchronization of cycles was attained in all three treated lots as may be seen in table 2. A higher percentage of MAP-fed cows were in estrus on days 3 and 4 than CAP-fed cows. The animals listed as "silent ovulators" include only those known to have ovulated without being recorded in estrus. Some of the cows listed as "others not in estrus during the synchronization period" returned to estrus approximately 22 days after the synchronization period and were probably "silent ovulators" or were in estrus and missed during the synchronization period.

Some of the animals that did not come in estrus during the synchronization period were in noticeably poor condition, or were quite old; most of these animals (a total of 8 treated and 7 controls) failed to come in estrus during the entire experimental period.

The conception rates obtained in each group are shown in table 2. The results are expressed as percentages of synchronized cows pregnant after one and two services and the percentage of all cows in each group pregnant after two services. The conception rate in the MAP-fed cows at the synchronized estrus (65.4) was highest; the conception in the CAP-fed and CAP-plus-thyroprotein-fed groups was similar to that obtained in the control group. The addition of thyroprotein to CAP did not appear to improve either synchronization or fertility.

Twelve of 29 control cows bred during the period before removal of the calves returned to estrus at shortened intervals (8-14 days, in most cases). This response is characteristic of a failure of the corpus luteum to form and function in a normal way; it has been produced experimentally by dilating or irritating the uterus and by oxytocin injections. Some stress associated with the calves, or their removal, may have contributed to this result.

Unfortunately, one of the seven bulls used in the experiment proved to be somewhat more fertile than the others and this bull was used almost exclusively in the MAP-fed group. The conception rates of the bulls used ranged from 44 to 76 percent. The differences in fertility of the MAP-fed cows and the other three groups are therefore confounded with differences in fertility of the bulls used. The conception rate for all natural services was 57.7 percent; for all artificial breedings it was 50.5 percent.

In the final trial (Experiment 6, table 2) the amount of CAP fed during the last 4 days of the feeding period was reduced from 10 to 6 mg. per animal, in an attempt to improve the synchronization and minimize the endometrial hyperplasia sometimes seen in the uteri of animals slaughtered 3 days after estrus following oral progestin treatment (Hansel, 1964, unpublished observations). Although these animals have not yet been checked for pregnancy, preliminary results based on nonreturns to estrus are encouraging.

The results obtained with sheep, summarized in table 3, show that a high degree of control of the estrous cycle can also be obtained by feeding MAP to cycling ewes. The poor results obtained in the one trial conducted with CAP (Expt. 9, table 3) were probably due to the fact that the compound was fed at too low a level, since many ewes came in estrus while on treatment.

The minimum effective level of MAP for ewes appears to be in the range 50-60 mg. per day, and the best fertility was obtained with treatment periods of 20 days. Reducing the daily dose to 30 mg. per day and the feeding period to 16 days (Expt. 2, table 3) resulted in less effective synchronization and lower conception rates. Cycling Dorset ewes were well synchronized when fed MAP in March (Expt. 3, table 3) and 76 percent of them conceived to first service.

The conception rates of cycling ewes bred at synchronized cycles following MAP feeding have been only slightly lower than conception rates in control ewes where natural breeding has been used. There appeared to be some advantage in breeding ewes as soon as they were found in estrus and again 12 hr. later, provided they would still accept the ram. In some (but not all) trials conception rates comparable to those obtained at natural matings have been obtained in ewes bred artificially with fresh semen diluted in physiological saline (Expts. 1 and 2, table 3). Uniformly low conception rates have been obtained in both control and synchronized ewes bred with extended semen stored for 24 hr. There is clearly a very real need to develop more satisfactory semen extenders and insemination techniques for sheep.

The combined use of MAP and PMS appears useful in inducing estrus and fertile ovulations in anestrous ewes (Expts. 4, 5, 6, 7, table 3). The relatively simple treatment consisting of 750 I.U. pregnant mare serum (PMS) on days 1 and 15 and 60 mg. MAP per day fed on days 7-14 of the treatment period seems particularly promising. This treatment also proved effective in lactating ewes (Expt. 8, table 3) and when used in ewes at the very beginning of the normal breeding season (Expt. 6, table 3).

Lambing rates of the synchronized ewes have not differed significantly from those of the untreated ewes, although the rates have been slightly higher in the PMS-treated ewes.

As was the case in cattle, the ewes failing to conceive at the synchronized estrus have returned to estrus after a normal cycle interval, have remained reasonably well synchronized, and have had essentially normal conception rates when bred at this time. A small number of ewes that returned to estrus and were rebred have lambed to the initial breeding.

Relatively few experiments on cycle regulation in swine have been reported since those of Nellor, (12). The high incidence of both follicular and luteal cysts in gilts following progestin feeding has been a serious deterrent to the development of effective cycle synchronization techniques in this species.

The trial with the progestin SC-10363 (Expt. 1, table 4) illustrates this point. A large proportion of the gilts fed the compound at the lower level and a smaller proportion of those fed it at the higher level developed cystic follicles.

The finding that exogenous estrogens can maintain swine corpora (Gardner et al., 5) suggested the possibility of synchronizing the estrous cycles of gilts by feeding an orally effective estrogen (MEE) for 9 days followed by an orally effective progestin (MAP) for a similar period. The results of the first trial (Expt. 2, table 4) were most encouraging; 9 of 11 gilts were synchronized and 7 of these farrowed when bred at the synchronized estrus. A more recent trial (Expt. 3, table 4) involving the same treatment has given less favorable results; only 14 of 20 gilts were synchronized and the synchronization period was long (days 4-10).

Many of these experiments have rather obvious shortcomings insofar as design and numbers of animals studied are involved but they have served several useful purposes in illustrating the types of compounds likely to be most useful, the approximate effective dose levels and feeding periods and methods of administration. Clearly, more carefully designed experiments involving larger numbers of animals are needed to evaluate the fertility of animals whose cycles have been synchronized by various methods.

The methods outlined certainly do not represent the only approaches to estrous cycle regulation, although a casual reader of the current literature might suppose that they do. Nonsteroidal compounds having antigonadotropic or antiestrogenic properties are available and should be tested for this purpose. Many other steroids, particularly those having maximum antigonadotropic properties and minimal uterotropic effects need to be tested for their ability to regulate the cycle. Other routes of administration need to be studied since a large proportion of the Nation's beef cattle are managed under conditions that make feeding the hormones impractical. Subcutaneous and intramuscular injections of potent progestins such as CAP and MAP apparently do not result in good cycle synchronization, although few reports on this subject are available. Finally, the possibility of regulating estrous cycles by antigonadotropins produced in response to gonadotropins injected as antigens as suggested by the work of Snook and Cole, (13) seems worthy of further investigation.

At present, our incomplete understanding of the luteotropic and luteolytic mechanisms in the various classes of farm animals constitutes a major deterrent to the development of more effective cycle regulation techniques. Theoretically, a treatment capable of maintaining corpora for a period of time (luteotropic) followed by one that would cause rapid regression of corpora (luteolytic) should result in a high degree of cycle synchronization.

Summary and Conclusions

Estrous cycle regulation studies have been carried out with 180 Holstein heifers, 555 Hereford and crossbred beef cattle, 816 cycling and anestrous ewes of several breeds, and 85 Yorkshire gilts. The results appear to justify the following conclusions:

(1) The orally active progestins, MAP and CAP, were both effective inhibitors of estrus and ovulation in cattle and sheep.

(2) Withdrawal of either compound from the feed resulted in effective estrous cycle synchronization in cattle and sheep. A high percentage of the treated animals came in estrus 3-6 days after hormone withdrawal.

(3) The minimal effective dose for cattle for MAP was approximately 200 mg. per animal per day, and for CAP it was approximately 10 mg. per animal per day. The comparable figure for MAP for ewes was 50-60 mg. per day.

(4) The optimal length of the hormone feeding period for both sheep and cattle was 18-20 days.

(5) Group feeding of either compound in grain or liquid rations gave satisfactory results.

(6) The conception rates of groups of Holstein heifers bred artificially at the first estrus after feeding MAP and CAP ranged from 48 to 70 percent. These rates were generally below those obtained in untreated heifers bred artificially under optimal conditions.

(7) Conception rates in both control and MAP- and CAP-treated beef animals bred artificially were somewhat lower. In one trial involving approximately 400 crossbred beef cows, conception rates to first service of approximately 40 percent were obtained for both control and CAP-fed cows, and 65 percent for MAP-fed cows.

(8) Conception rates of cycling ewes bred naturally at synchronized cycles following

MAP feeding were slightly lower than rates obtained in control ewes.

(9) Dairy heifers, beef cattle, and cycling ewes failing to conceive at the synchronized estrus returned to estrus after normal cycle intervals and apparently normal conception rates were obtained at the second breedings.

(10) Synchronous estrous cycles and ovulations were induced in anestrous and anestrous lactating ewes with a combined PMS (750 I.U. injected on days 1 and 15) and MAP (60 mg. per day fed on days 7-14) treatment. Conception rates to first service of 57 to 85 percent were obtained in ewes synchronized in this way.

(11) A sequential treatment involving estrogen (MEE for 9 days) and progestin (MAP for 9 days) resulted in good synchronization and fertility in one group of gilts, but did not give good synchronization in a second group.

Acknowledgment

The work discussed could not have been accomplished without the cooperation and financial assistance of many organizations and individuals. The cooperative efforts of D. E. Hogue, W. G. Wagner, R. W. Bratton, W. Pond, R. H. Foote, P. V. Malven, M. A. Brunner, H. VanBlake, D. L. Black, R. S. Newman, D. G. Beresford, R. L. Kittle, H. F. Ley, and G. L. Hunt are gratefully acknowledged. The financial support and other assistance furnished by the National Association of Artificial Breeders, The New York Artificial Breeders Cooperative, The Upjohn Co., Eli Lilly and Co., Briarcliff Farms Inc., and Syntex Laboratories, Inc., are also gratefully acknowledged. The experiments were also supported, in part, by the regional research project NE-41 entitled Endocrine Factors Affecting Reproduction in Dairy Cattle, a cooperative study by agricultural experiment stations in the northeast and the Dairy Husbandry Research Branch, Agriculture Research Service, U.S. Department of Agriculture.

Literature Cited

(1) Anderson, L. L., Schultz, J. R., and Melampy, R. M.
1964. Pharmacological Control of Ovarian Function and Estrus in Domestic Animals. Gonadotropins: Their chemical and biological properties and secretory control. Ed. H. H. Cole. W. H. Freeman and Co., San Francisco. Sixth Animal Reproduction Symposium Proc., Aug. 1963, Oreg. State Univ., Corvallis.

(2) Brunner, M. A., Hansel, W., and Hogue, D. E.
1964. Use of 6-Methyl-17-Acetoxyprogesterone and Pregnant Mare Serum to Induce and Synchronize Estrus in Ewes. Jour. Anim. Sci. 23: 32.

(3) Evans, J. S., Dutt, R. H., and Simpson, C. C.
1962. Breeding Performance in Ewes after Synchronizing Estrus by Feeding 6-Methyl-17-Acetoxyprogesterone. Jour. Anim. Sci. 21: 804.

(4) Foote, R. H., Gray, Linda C., and Young, D.C.
1960. Fertility of Bull Semen Stored up to Four Days at 5° C in 20 percent Egg Yolk Extenders. Jour. Dairy Sci. 43: 1330.

(5) Gardner, M. L., First, N. L., and Casida, L. E.
1963. The Effect of Exogenous Estrogens on Corps Luteum Maintenance in Gilts. Jour. Anim. Sci. 22: 132.

(6) Hansel, W.
1961. Estrous Cycle and Ovulation Control in Cattle. Jour. Dairy Sci. 44: 2307.

(7) Hansel, W., Malven, P. V., and Black, D. L.
1961. Estrous Cycle Regulation in the Bovine. Jour. Anim. Sci. 20: 621.

(8) Hansel, W., Van Blake, H., and Malven, P. V.
1962. Artificial Estrous Cycle Synchronization in Cattle by Feeding Techniques. Cornell Nutrition Conf. Proc., Sept. Ithaca, N. Y.

(9) Hansel, W. and Wagner, W. C.
1960. Luteal Inhibition in the Bovine as a Result of Oxytocin Injections, Uterine Dilatation and Intrauterine Infusions of Seminal and Preputial Fluids. Jour. Dairy Sci. 43: 796.

(10) Hartman, C. G. (ed.)
1963. Mechanisms Concerned with Conception. Conf. on Physiological Mechanisms Concerned with Conception. Proc., July 1959, West Point, N. Y. The MacMillan Co., New York.

(11) Hogue, D. E., Hansel, W., and Bratton, R. W.
1962. Fertility of Ewes Bred Naturally and Artificially after Estrous Cycle Synchronization with an Oral Progestational Agent. Jour. Anim. Sci. 21: 625.

(12) Nellor, J. E.
1960. Control of Estrus and Ovulation

in Gilts by Orally Effective Progestational Compounds. Jour. Anim. Sci. 19: 412.

(13) Snook, R. and Cole, H. H.
1964. Endogenous Gonadotropic Activity in Mare Serum Subsequent to Chronic Treatment with Gonadotropin. Endocrinology 74: 52.

(14) Southcott, W. H., Braden, A. W. H., and Moule, G. R.
1962. Synchronization of Oestrus in Sheep by an Orally Active Progesterone Derivative. Aust. Jour. Agr. Sci. 13: 901.

(15) Van Blake, H., Brunner, M. A., and Hansel, William
1963. Use of 6-Chloro- Δ^6- Dehydro-17-Acetoxyprogesterone (CAP) in Estrous Cycle Synchronization in Diary Cattle. Jour. Dairy Sci. 46: 459.

Table 1. Results of estrous cycle synchronization trials with orally active progestins in Holstein heifers

Experiment and date	Aminals used and treatment^{ab}	No.	No. synchronized	No. of "silent" ovulators	Others not in estrus during synchronization period	Synchronized animals		All animals	
						Pregnant to first service (%)	Pregnant after two services (%)	Pregnant to first service (%)	Pregnant after two services (%)
1. (1962)	Untreated	20	-	-	-	-	-	65	80
2. (1960)	MAP-0.25-1.0 mg per lb. on days 14-22 of the estrous cycle. (preliminary trials)	7	5(2-5)	1	1	-	-	-	-
3. (1961)	MAP^{c}-0.5 mg per lb. for 20 days and 0.05 mg per lb. for an additional 6 days.	20	18(3-4)^{e}	0	2	55	-	50	-
4. (1961)	MAP-0.4 mg per lb. for 10 days and 0.05 mg per lb. for an additional 5 days^{f}.	15	15(3-5)	0	0	60	-	60	-
5. (1962)	MAP-240 mg per heifer for 14 days.	19	18(2-6)	1	0	50	-	47	90
6. (1961)	CAP^{d}-0.3 mg per lb. for 20 days.	4	2(13)	2	0	0	-	0	-
7. (1961)	CAP-0.1 mg per lb. for 20 days.	4	4(6-8)	0	0	50	-	50	-
8. (1961)	CAP-0.05 mg per lb. for 15 days.	4	4(5-7)	0	0	100^{g}	-	100	-
9. (1962)	CAP-0.025 mg per lb. for 15 days.	4	4(4-7)	0	0	75^{h}	-	75	-

TABLE 1. Results of estrous cycle synchronization trials with orally active progestins in Holstein heifers (Continued)

Experiment and date	Animals used and treatment[ab]	No.	No. synchronized	No. of "silent" ovulators	Others not in estrus during synchronization period	Synchronized animals		All animals	
						Pregnant to first service (%)	Pregnant after two services (%)	Pregnant to first service (%)	Pregnant after two services (%)
10. (1962)	CAP-0.02 mg per lb. for 20 days..	20	18(4-6)	1	1	53[i]	-	50	-
11. (1962)	CAP-12 mg per heifer per day for 18 days.	25	23(6-9)	0	2	70	95	68	92
12. (1962)	CAP-12 mg per animal per day. 3 yr. old lactating cows.	8	7(3-7)	0	1	57	-	50	-
13. (1963)	CAP-10 mg per heifer per day.	30	27(3-6)	-	3[j]	48	63	50	60
Total	Treated Controls	160 20	145	5	10				

[a] All animals were bred artificially with semen supplied by the New York Artificial Breeders Cooperative.
[b] All animals were individually fed except those in experiments 11 and 13 which were group fed.
[c] MAP = 6-methyl-17-acetoxyprogesterone (The Upjohn Co.)
[d] CAP = 6-chloro- Δ^{6}-dehydro-17-acetoxyprogesterone (Eli Lilly and Co.)
[e] Numbers in parentheses indicate range in days after hormone dose lowered or hormone withdrawn.
[f] Self-retaining catheters were placed in the uteri of two heifers that were within the first 5 days of their cycles at the beginning of the trial. All heifers received single subcutaneous injections of 100 USP units of oxytocin at the beginning of the hormone feeding period.
[g] One heifer aborted at the 69th day.
[h] One heifer checked pregnant returned to estrus at 55 days.
[i] One heifer checked pregnant later returned to estrus.
[j] All 3 heifers in estrus on day 11.

Table 2. Results of estrous cycle synchronization trials with orally active progestins in beef heifers and cows

Experiment and date	Animals used and treatments[a]	No.	No. synchronized	No. of "silent" ovulators	Others not in estrus during synchronization period	Synchronized animals[1]		All animals	
						Pregnant to first service (%)	Pregnant after two services (%)	Pregnant to first service (%)	Pregnant[1] after two services (%)
1. (1960)	MAP[b]-968 mg per animal per day for 10 days followed by 500 mg per day for 10 days. Mature Hereford cows with calves.	32	16(3-5)[c]	13	3	31	-	25	-
2. (1960)	Enovid[b]-187 mg per animal per day for 30 days. Mature Hereford cows with calves.	18	2(2-5)[d]	-	16	-	-	-	-
3. (1961)	A. Untreated Hereford heifers.	20		-	11[e]	-	-	15	25
	B. MAP-0.4 mg per lb. for 19 days followed by 0.05 mg per lb. for 5 days. Hereford heifers.	20	19(4-7)	-	1	37	63	35	60
4. (1961)	A. Untreated mature Hereford cows with calves.	15	-	-	7[e]	-	-	33	-
	B. MAP-400 mg per animal per day for 10 days and 50 mg per day for an additional 5 days. Mature Hereford cows with calves[f].	35	8(3-7)	10	17	12	-	17[g]	-
5. (1963)	A. MAP-240 mg per animal per day for 18 days. Mature crossbred cows; calves removed.	96	81(2-8)	3	12	65	88	56.5	80[hi]
	B. CAP[b]-10 mg per animal per day for 19 days. Mature crossbred cows; calves removed.	98	90(3-8)	4	4	36	70.0	33	70[j]

Table 2. Results of estrous cycle synchronization trials with orally active progestins in beef heifers and cows (Continued)

Experiment and date	Animals used and treatments[a]	No.	No. synchronized	No. of "silent" ovulators	Others not in estrus during synchronization period	Synchronized animals[1]		All animals	
						Pregnant to first service (%)	Pregnant after two services (%)	Pregnant to first service (%)	Pregnant[1] after two services (%)
	C. CAP-10 mg per animal per day and thyroprotein 9 mg per day for 19 days. Mature crossbred cows; calves removed.	97	80(3-9)	9	8	41	70.0	34	68[k]
	D. Untreated mature crossbred cows; calves removed.	95	-	-	7[e]	-	-	39	70
6. 1964	CAP-10 mg per animal per day for 14 days followed by 6 mg per day for 4 days. Mature Hereford cows with calves.	29	23	4	2	61[m]	-	65[m]	-
Total	Treated	425	319	43	63				
	Controls	130							

a All animals were group fed. The progestins were incorporated in amounts of grain equivalent to 3 to 5 lbs. per animal per day.

b MAP = 6-methyl-17-acetoxyprogesterone (The Upjohn Co.). CAP = 6-chloro- Δ^6-dehydro-17-acetoxyprogesterone (Eli Lilly and Co.). Enovid = 17-ethynyl-17-hydroxy-estr-5(10)-en-3-one containing 1.5% 3-methyl ether of ethynyl estradiol (G. D. Searle and Co.).

c Numbers in parentheses represent range in days after hormone withdrawal except in Experiments 3 and 4 where they represent days after the dose was lowered.

d Eleven cows came in estrus during the hormone feeding period.

e Not in estrus during the 20-day experimental period.

f Self-retaining catheters were placed in the uteri of 16 of the 35 cows at the beginning of the feeding period.

g Five of 23 cows bred without showing estrus conceived.

h Includes 9 cows not in estrus during the synchronization period that came in estrus and were bred (for the first time) one cycle later.

i Includes one cow pregnant to insemination after "silent" ovulation on day 5 of the synchronization period.

j Includes 6 cows to which footnote h applies.

k Includes 12 cows to which footnote h applies.

l All animals were bred artificially except in Experiment 5 in which half were bred artificially and half naturally.

m Based on 30-day nonreturns.

Table 3. Results of estrous cycle synchronization trials with orally active progestins in sheep

Experiment and date	Animals used and treatments[a]	No. of ewes	No. synchronized	No. bred	30-day non-returns (%)	Lambing to first breeding (%)	Lambing to first or second breeding (%)	Lambing rate (first breeding only) (%)
1. Fall-1960	Cycling western white-face ewes.							
	A. Untreated. Bred naturally.	40	-	40	58	50	94	-
	B. Untreated. Bred artificially.[b]	39	-	39	59	49		
	C. MAP-60 mg per day for 20 days. Bred naturally.	20	18(2-3)[c]	18	44	61	92	-
	D. MAP-60 mg per ewe per day for 20 days. Bred artificially.[b]	20	19(2-3)	19	21	16		
2. Fall-1961	Cycling western white-face ewes.							
	A. Untreated. Bred naturally.	16	-	16	81	75	94	158
	B. Untreated. Bred artificially with extended semen.[d]	14	-	14	64	43	80	183
	C. Untreated. Bred artificially with stored extended semen.[e]	15	-	15	20	7	87	100

Table 3. Results of estrous cycle synchronization trials with orally active progestins in sheep (Continued)

Experiment and date	Animals used and treatments[a]	No. of ewes	No. synchronized	No. bred	30-day non-returns (%)	Lambing to first breeding (%)	Lambing to first or second breeding (%)	Lambing rate (first breeding only) (%)
2. Fall-1961 (continued)	D. MAP-60 mg per ewe per day for 20 days. Bred naturally.	15 45	43(2-3)	13	62	46	77	167
	E. MAP-60 mg per ewe per day for 20 days. Bred artificially with extended semen.[d]	15		15	40	33	93	100
	F. MAP-60 mg per ewe per day for 20 days. Bred artificially with stored extended semen.[e]	15		15	13	7	80	200
	G. MAP-30 mg per ewe per day for 16 days. Bred naturally.	15 45	36(2-4)	6	50	50	100	133
	H. MAP-30 mg per ewe per day for 16 days. Bred artificially with fresh extended semen.[d]	15		15	0	13	80	100
	I. MAP-30 mg per ewe per day for 16 days. Bred artificially with stored extended semen.[e]	15		15	7	0	100	-
3. March-1962	Cycling Dorset ewes							
	A. Untreated. Bred naturally.	44	-	43	79	77	86	121
	B. MAP-60 mg per ewe per day for 20 days. Bred naturally.	42	34(3-5)	34	80	76	91	129
4. May-1961	Anestrous Hampshire and Corriedale ewes							
	A. Untreated	15	0	0		-	-	-

Table 3. Results of estrous cycle synchronization trials with orally active progestins in sheep (Continued)

Experiment and date	Animals used and treatments^a	No. of ewes	No. synchronized	No. bred	30-day non-returns (%)	Lambing to first breeding (%)	Lambing to first or second breeding (%)	Lambing rate (first breeding only) (%)
4. May-1961 (continued)	B. MAP-60 mg per ewe per day for 20 days.	15	0	0		-	-	-
	C. MAP-60 mg per ewe days 1-8 and 15-22; 750 I.U. PMS days 9-23.^{f}	43	33(2-6)	33	-	64	-	157
5. May-1962 and 1963	Anestrous Hampshire and Corriedale ewes							
	A. Untreated.	20	0	1	-	0	-	-
	B. MAP-50 mg per ewe days 1-8 and 16-23; 750 I.U. PMS days 9 and 24. Bred naturally.	28 56	44(2-6)	19^h	-	58	-	155
	C. MAP-50 mg per ewe days 1-8 and 16-23; 750 I.U. PMS days 9 and 24. Bred artificially.^{g}	28		21^h	-	57	-	158
6. July-Aug.-1962	Hampshire and Corriedale ewes							
	A. MAP-60 mg per ewe for 20 days. Bred naturally.	16	13(2-6)	13	62	62	-	138
	B. MAP-60 mg per ewe days 1-8; 750 I.U. PMS day 9. Bred naturally.	16	14(2-6)	14	64	57	-	163
	C. 750 I.U. PMS day 1; MAP-60 mg per ewe days 7-14. Bred naturally.	16	13(2-6)	13	62	69	-	122
	D. 750 I.U. PMS days 1 and 15; MAP-60 mg per ewe days 7-14. Bred naturally.	17	17(2-8)	17	82	71	-	142

Table 3. Results of estrous cycle synchronization trials with orally active progestins in sheep (Continued)

Experiment and date	Animals used and treatments[a]	No. of ewes	No. synchronized	No. bred	30-day non-returns (%)	Lambing to first breeding (%)	Lambing to first or second breeding (%)	Lambing rate (first breeding only) (%)
7. July-Aug.-1963	Hampshire and Corriedale ewes[l]							
	A. Untreated. Bred naturally.	29	-	29	86	83	90	158
	B. 750 I.U. PMS days 1 and 15; MAP-60 mg per ewe days 7-14. Bred naturally.	73	63(2-7)	63	67	63	89	143
8. May-1963	Lactating western whiteface ewes							
	A. Untreated.	17	0	0	-	-	-	
	B. MAP-60 mg per ewe 20 days.	17	0	0	-	-	-	
	C. 750 I.U. PMS days 1 and 15; MAP-60 mg per ewe days 7-14.	17	13(2-4)	13	85	85	-	155
9. Fall-1962	Cycling western whiteface ewes							
	A. MAP-60 mg per ewe for 18 days. Bred naturally.	20	19(3-4)	19	53	-	-	-
	B. CAP-0.5 mg per ewe for 18 days. Bred naturally.	30	16(4-5)[i]	16	19	-	-	-
	C. CAP-1.0 mg per ewe for 18 days. Bred naturally.	30	20(4-7)[j]	20	30	-	-	-
	D. CAP-2.0 mg per ewe 18 days. Bred naturally.	29	18(4-7)[k]	18	44	-	-	-
Total	Treated Controls	567 249						

---

[a] All animals were group fed. The progestins were incorporated into an amount of soybean oil meal equivalent to 0.2 lb. per ewe per day.

[b] Inseminated intracervically with 0.5 ml fresh semen diluted with physiological saline and containing $50\text{-}870\times10^6$ motile sperm per ml.

[c] Numbers in parentheses indicate range in days after hormone withdrawal.

[d] Extended in physiological saline to 10×10^7 motile cells per ml; 0.5 ml per insemination.

[e] Extended in CUE (Foote et al., 1960) and stored for 24 hrs.

[f] All PMS was obtained from Boots Pure Drug Co., Nottingham and was injected subcutaneously.

[g] Extended in physiological saline to contain 30 to 40×10^7 motile cells per ml; 0.5 ml per insemination.

[h] Four synchronized ewes lost; one was sold and one died without pregnancy diagnoses, two stood for teaser rams, but would not stand for fertile rams later in the day.

[i] Fifteen ewes came in estrus while on treatment.

[j] Five ewes came in estrus while on treatment.

[k] Nineteen ewes came in estrus while on treatment.

[l] Treatments effects confounded with ram effects.

Table 4. Results of estrous cycle synchronization studies with oral progestins in swine

Experiment and date	Animals used and treatments[a]	No. of gilts	No. synchronized	No. bred	Conceived to first service (%)	Conceived to first or second service (%)	Litter size
1. (1961)	Yorkshire gilts						
	A. Untreated controls. Bred artificially.[g]	11	-	10	(50)[h]	100	8.7
	B. SC-10363[b]-0.3 mg per lb. for 15 days. Bred artificially.	11	10(2-6)[l]	10	0[i]	100	10.2
	C. SC-10363-0.5 mg per lb. for 15 days. Bred artificially.	11	11(2-6)	11	27[j]	91	7.8
2. (1962)	Yorkshire gilts						
	A. Untreated controls. Bred naturally.	11	-	11	82[k]	100	8.1
	B. MEE[c]-16 mg per gilt for 9 days followed by MAP 0.6 mg per lb. for 9 days. Bred naturally	11	9(3-8)	9[e]	78[k]	89	8.9
3. (1964)	Yorkshire gilts						
	A. Untreated controls. Bred naturally.	10	-	9			
	B. MEE-16 mg per gilt for 9 days followed by MAP-0.6 mg per lb. for 9 days. Bred naturally.	20	14(4-10)	14[f]	-	-	-

[a] All hormones fed in 3 or 4 lbs. of a typical swine gestation ration fed twice daily.

[b] SC-10363 = orally active progestin (The G. D. Searle Co.).

[c] MEE = 3 methyl ether of ethynyl estradiol (Eli Lilly and Co.).

[d] MAP = 6-methyl-17-acetoxyprogesterone (The Upjohn Co.).

[e] One gilt in estrus on day 8 of MEE feeding; one of the 2 gilts not showing estrus during the synchronization period remained anestrous for 5 months and was found to have large cystic follicles on both ovaries at slaughter.

[f] One gilt came in estrus on the 5th day of MAP feeding and again 19 days later.

[g] Each gilt inseminated with 50 ml of semen diluted in CUE extender to contain 3 billion motile sperm (Foote et al., 1960).

[h] Five gilts in each treated group and 4 controls were slaughtered 2-4 days after breeding and ova recovered. The conception data are based on nonreturns and fertilized ova. Litter sizes are based on the 6 remaining animals in each group.

[i] Four of 5 gilts slaughtered at 2-4 days after breeding had cystic follicles. The remaining one had inactive ovaries.

[j] One of 5 gilts slaughtered at 2-4 days after breeding had cystic follicles.

[k] Based on numbers farrowing.

[l] Numbers in parentheses indicate range in days after hormone withdrawal.

EVALUATION OF SOME METHODS FOR CONTROLLING THE BOVINE ESTROUS CYCLE

R. G. Zimbelman
Veterinary Division, The Upjohn Company, Kalamazoo, Michigan

Introduction

This paper will deal primarily with the evaluation of various methods to control the estrous cycle in cattle. Consideration will be given to four methods of altering the normal estrual cycle. (A) The inhibition of ovulation as a means of altering the estrual cycle appears to be receiving most attention in recent years. The bulk of this paper will deal with ovulation inhibition by progestogens; thus, it would seem appropriate to give a brief evaluation of the other three methods.

(B) Ovulation induction by various means has been so thoroughly studied in the last 25 to 30 years that no additional comments are really necessary at this time. Induction of ovulation during the luteal phase of the estrous cycle has not been accompanied by production of fertilizable ova (Hansel, 5). Few new data have been added to change previous concepts. The use of hypothalamic factors which cause release of endogenous pituitary hormones represents a potential new approach with many intriguing possibilities. Additional information is needed in this area for adequate evaluation.

(C) Inducing or delaying regression of the corpus luteum also offers an interesting approach to estrous cycle control. The best model for regression would be manual removal of the corpus luteum. This procedure has obvious practical shortcomings which have been discussed before (Hansel, 5). Also, the effect of oxytocin during a specific period of corpus luteum formation in the bovine could be included here as an example of an agent altering luteal development. We need to find an agent which is effective at all stages of the estrual cycle before this method can be seriously considered.

(D) Another potential method of controlling the estrous cycle would be to alter the developmental pattern of the heifer so that ovulation would no longer occur spontaneously. A condition sometimes termed "constant estrus" has been produced in female rats by the administration of androgens within the first 5 days after birth (Barraclough and Gorski, 2). The induction of ovulation in a predictable manner could conceivably result in synchronization of ovulation. Our knowledge is inadequate in this area and many questions need to be answered before this approach can be fairly evaluated. Newborn calves and pigs have been injected with androgens within 48 hours after birth (Hansel, 10; Zimbelman, 11), but without results like those obtained from studies of rats.

The balance of this paper will relate some data on ovulation inhibition in cattle with the goal of emphasizing certain guidelines to be used in evaluation of this method to control the estrous cycle.

Experimental Methods

Studies involving dairy heifers were begun only after determining that the heifers were sexually mature and experiencing estrous cycles of normal length. Twice daily observations for estrus and regular rectal palpation of the genital tract were used to determine the influence of treatment. Data on heifers were usually collected under two general procedures: (1) Individual feeding of a compound to dairy heifers for 16 days beginning at the 15th day of the estrous cycle. (2) Group feeding of heifers beginning on the same day, with animals in various stages of the estrous cycle. Oral administration of the progestogens was accomplished by feeding the compound in 1 to 4 pounds of grain per animal daily either at single or divided feedings. Single injections of MAP were made into the ear in volumes up to 5 ml. per ear.

Studies in post-partum cows involved placing cows in their proper group each week at 2 intervals from calving, either 9 to 15 or 16 to 22 days. Animals were randomly assigned to the various experimental groups. Only cows nursing a calf throughout the study were included in the results. The cows were fed 4 pounds of grain daily during the feeding period of 10 or 17 days. After the feeding period, the cows were put to pasture with two bulls. Observations for estrus were made twice daily throughout and after the feeding period until the cows were diagnosed pregnant. Rectal palpations were performed weekly to determine ovarian condition and for pregnancy diagnosis.

Statistical analyses were performed using standard tests described in statistical texts. More detailed descriptions of methods used have been published previously (Zimbelman, 9).

Results and Discussion

Minimal Effective Dosages for Some Compounds

In an earlier publication, dosage titration of medroxyprogesterone acetate (MAP)[1] in heifers was reported (Zimbelman, 9). The minimal effective oral dose was shown to be on the order of 180 mg. daily. Additional studies by other workers and field trials have confirmed that this dosage is effective in beef heifers and cows. Another compound of interest is chlormadinone acetate (CAP) and the minimal effective dose of CAP would appear to be on the order of 5 to 10 mg. daily based on other reports (VanBlake *et al.*, 7; Wagner *et al.*, 8).

---

[1] Repromix, ® The Upjohn Company.

Degree of Synchronization Following Inhibition of Ovulation

The degree of synchronization following MAP has also been described in numerous reports. Our data indicated that 95% of the beef and dairy heifers observed in estrus were observed on the second, third, or fourth days after last feeding of MAP at doses in the range of 120 to 400 mg. daily (Zimbelman, 9). Longer intervals from last feeding to first estrus following CAP have been reported (VanBlake *et al.*, 7). This would seem to indicate that either CAP is being used at doses considerably greater than minimal or that the compound acts differently. The answer can come only from simultaneous comparisons at a wide range of doses, some of which are too low to inhibit ovulation.

Conception Rates Following Estrus Synchronization With MAP

The conception rate of animals sychronized with MAP varied from 26 to 60% at the synchronized estrus (S1) in various beef heifer trials (table 1). A consistently high conception rate was noted at the second cycle (S2) of synchronized heifers. A similar trend was found in studies in beef cows over a 2-year period (table 2). The conception rate after two services (S1 + S2) remained quite high regardless of that encountered at the synchronized estrus. Since synchronization with MAP allowed most animals the opportunity to be bred twice in a 25- to 26-day period, the conception rate within the first 26-day period (S26) after last feeding of MAP was calculated to be 77% of the treated animals. The difference between 77% conceiving within 26 days and 89% conceiving with two services was due to two reasons: (1) Failures to detect estrus and, thus, to reinseminate at the second cycle were responsible for about two-thirds of the difference, and (2) in about one-third of the cycles in question, the second ovulations were later than 26 days after last feeding. The fact that conception rates seem to vary markedly from trial to trial conducted in similar fashion should be given consideration by each person planning research on estrous synchronization. Any comparisons of types of animals studied, frequency of feeding, dosage effects, etc., should be made simultaneously. Any deviation from completely contemporaneous comparisons makes the results subject to question.

Effect of MAP on Post-Partum Intervals

The effect of MAP on post-partum intervals in cows was given attention because of its unique importance to successful synchronization. Many cows that are candidates for estrous synchronization may not have returned to normal estrous cycle patterns. Data are available from two trials, one in 1962 with first-calf heifers and one in 1963 with essentially the same group of animals after second calving. The results of the 1962 study were reported earlier (Zimbelman, 9), but are repeated for comparison. The number of animals involved and designs of the trials in 1962 and 1963 are shown in table 3. Basically, the study involved placing cows on trial once each week beginning at two intervals from calving, either 9 to 15 or 16 to 22 days, and feeding for 10 or 17 days. Table 4 shows the average intervals from calving to first post-treatment ovulation and to conception. There were no statistically significant differences in these intervals. However, the relatively short intervals of about 37 and 33 days for cows studied at the early interval from calving and the tendency for treated animals to have shorter intervals to ovulation than control animals are noteworthy. Some untreated cows appeared to have ovulated during treatment, but the average contains only data for the first post-treatment ovulation (table 6).

It should also be stated that we have concluded that rectal palpations of post-partum beef cows do not appear to be nearly as reliable as in cycling dairy heifers. The greatest aid to our study was having fertile bulls present for estrus detection and breeding. A combination of accurate pregnancy diagnosis at 30 to 40 days of gestation, of estrus detection records, and rechecking of weekly ovarian measurements usually allowed us to establish the day of ovulation rather closely. However, any single criterion alone was inadequate.

The average interval from last MAP feeding to next ovulation for treated cows of 4.3 days in 1962 was significantly shorter ($P \cong .05$) than that for the control cows of 8.2 days (table 5). In 1963, the average of 5.3 days for 51 treated cows was significantly shorter ($P < .05$) than the average of 8.9 days for 17 control cows. In addition, the variation in intervals to first post-treatment ovulation was also reduced by treatment in both years. Both the reduction in average interval and in variance are characteristic of a synchronized estrus.

In table 6 the distributions of first post-treatment ovulation with respect to last feeding are shown. In the 2 years, 79% of 73 treated cows ovulated from 2 to 6 days after last feeding compared to 34% of 35 control cows. The control groups had more cows with both shorter and longer intervals. The distributions were significantly different when tested by Chi-Square ($P < .005$). Seven of the 15 control cows in estrus from 7 to 20 days after last feeding had apparently ovulated during the feeding period.

The conception rates for both groups of control cows for 1962 were pooled (table 7). In 1962, the conception rate at first cycle was similar for both treated and control cows, but it was somewhat lower in 1963 for treated cows. These conception rates are based on the assumption that each new ovulation was accompanied by a mating whether or not the mating was observed. These data show that variations in conception rate at the synchronized estrus occur with natural mating as well as with artificial insemination. The conception rate at second service was uniformly high in 1963; thus, the percentage conceiving at two cycles was quite similar in both years between treated and control groups. A higher proportion of treated cows (81% - both years) had conceived by 26 days after last MAP feeding than of control cows (71% - both years). In light of present recommendations, it also seems significant to point out that 79% of all cows had conceived by 59 days post-partum (table 8). This finding would seem to call for a reevaluation of the recommendation that cows not be bred until 60 days post-partum. Of course, it could be argued that the cows used in these studies are not typical of range beef cows. However, it might be fair to assume that these cows at least represent some sort of goal which might be obtained in the efficiency of beef cows if they were receiving proper management and nutrition. These data should not be interpreted as denying that some beef cows under other circumstances may have intervals to first ovulation of 90 days or more.

The data in table 9 illustrate that the interval from calving to conception of about 47 days was confirmed by the average calving interval of 329 days. The average gestation length of 282 days is within the normal range for this breed and is similar to the figure for the previous year for the same animals bred by artificial insemination (Zimbelman, 9). In summary, beef cows were synchronized by beginning treatment with 180 mg. MAP prior to the time that normal ovulation occurred. Additional studies need to be made in cows with longer intervals to ovulation than those of the untreated cows of these studies (that is, 37 days). Foote and Hunter (4) have reported similar findings in a study of the effect of daily injections of progesterone in post-partum beef cows. These workers pointed out that the form of progesterone administration seems important. Another study with a progesterone suspension administered as a single injection delayed rather than shortened the interval to first ovulation (Foote et al., 3). Any oral progestogen to be used in synchronizing estrus in range beef cows should be studied to determine its effect on the interval to first post-partum ovulation. It would seem justified to assume that different results might be obtained from different compounds or from different doses of the same compounds.

Effects of Single Subcutaneous Injections of MAP

In table 10 some data from two trials in which beef heifers received a single subcutaneous injection of MAP in the ear are summarized. In trial A, at 126 days after injection the heifers were slaughtered and the MAP was removed from the ear for chemical analysis. The mean amount of MAP recovered was 21 mg. from 11 heifers receiving 100 mg. and 76 mg. from 13 heifers that had received 500 mg. The average amount absorbed daily was 0.62 and 3.37 mg. for these two doses. Additional data to be presented later will show that the compound was still physiologically active at this time in some heifers (table 11). There were 20 heifers per group in trial B; 5 of each group were slaughtered in four different times from injection varying from 106 to 117 days. The average amount absorbed daily from 100 mg. injection in trial B was only 0.48 mg. daily. Thus, we can conclude that much less than 180 mg. daily is required if the compound is administered in an appropriate manner. Daily injections of 5 mg. intravenously have also proven adequate to inhibit ovulation (Zimbelman, 11). These data demonstrate that MAP is a very insoluble material and would seem to make daily intramuscular or subcutaneous administration a poor means of comparing potency.

The summary of reproductive performance during these studies is shown in table 11. Only one heifer which had received 500 mg. had ovulated at 126 days after injection. About 65% of the heifers receiving 100 mg. MAP begun to ovulate by 106 or more days after injection, as indicated by the presence of new corpora lutea at slaugher. The term "old corpora lutea" refers to those judged to be of a recent ovulation, but earlier than the one responsible for the corpora lutea termed "new." The increased incidence of old corpora lutea in trial B represented less complete inhibition of ovulation by 100 mg. than in trial A. Thus, the effect of single injection varied considerable between heifers within a given trial. Some heifers receiving 100 mg. had not ovulated recently while others had one or two sets of recent corpora lutea. The incidence of palpable corpora lutea also indicated that the effect of 100 mg. varied widely between trials as well, much greater effects being achieved in trial A than in trial B. Essentially no animals ovulated in trial A for 10 to 12 weeks after injection, while some animals in trial B never stopped ovulating. Thus, the chances of achieving synchronization of estrous cycles following a single injection seem rather remote. Rectal palpation at intervals of about every 2 weeks revealed an increased incidence and size of detectable follicles due to MAP treatment.

Characterization of Follicular Activity During Progestogen Influence

The follicular fluid weights of heifers receiving single injections of MAP are shown in table 12. These data were obtained by weighing the ovary intact, and again after slicing at 5 mm. intervals. In trial A, there was a significant increase in follicular fluid due to treatment. That there was more follicular fluid at 500 mg. appears to be due to the greater number of follicles since more heifers from the 100 mg. group had ovulated recently. The failure to find a significant increase in follicular fluid in trial B would appear to be due to the return to normal cyclic ovulation by most treated heifers by the end of trial B. Follicular fluid weights were also increased about threefold (1.33 vs. 4.01 gm.) during daily oral treatment with a progestogen at a dosage adequate to inhibit ovulation (Zimbelman, 11).

Evidences of Estrogenic Influence

It has been pointed out before that vulvar swelling, increased uterine tone, and frequent discharges of mucus occur in heifers during the period of ovulation inhibition with an oral progestogen (Zimbelman, 9). These signs are typical of an estrogenic influence. Cervical mucus smears from heifers which received a progestogen orally beginning at various stages of the estrual cycle were studied in accord with the procedures of Alliston et al., (1). Briefly, ratings of 1 and 2 represent little or no fern pattern, rating of 3 and 4 represent typical patterns over less than the complete smear, while ratings of 5 and 6 represent typical patterns over the entire smear. These smears were classified as coming from heifers in which the corpus luteum was or was not regressed. Regression was defined as reduction of 4 mm. in estimated size or disappearance of the corpus luteum found on previous palpation. After regression of the corpus luteum, 72% of the smears had a typical fern pattern as compared to only 17% when the mature corpus luteum was present (table 13). This difference was statistically significant ($P < .005$). Thus, oral progestogens may prevent ovulation as effectively as does the corpus luteum, but there appeared to be a definite estrogenic influence that was not detected in the luteal phase. The means by which the corpus luteum inhibits ovulation needs to be clarified. After regression of the corpus luteum, there was no difficulty in penetration of the cervix; this also is more typical of the estrual than of the luteal phase. The evidence seems to indicate a high degree of follicular stimulation both in terms of follicle size and of estrogenicity. If there is any basis for combination of progestogens and estrogens for estrous cycle control in cattle, it would not seem to be due to an estrogen deficiency under usual conditions.

Agents Which Will Induce Ovulation

One partial test of the mechanism of ovulation inhibition could be made by determining agents which will overcome the progestogen-induced inhibition. To carry out studies of this nature, heifers received a progestogen orally from day 15 after estrus until one week after injection of a possible ovulation inducer. Preliminary studies revealed that several estrogens were effective, the results varying with the route of administration and the vehicle used. Several gonadotropins appeared to be effective. Agents which failed to induce ovulation were: oxytocin (200 I.U.), amphetamine (300 mg.), and neostigmine (50 mg.). Ovulation was induced in 16 heifers each with an intravenous injection of either: 10 mg. estradiol cyclopentylpropionate, 5,000 I. U. of Chorionic Gonadotropin,[2] or 50 mg. of pituitary luteinizing hormone.[3] Based on gross appearance, the corpora lutea corresponding to days 1 and 4 of the estrous cycle seemed normal. However, at stages corresponding to days 7 and 10 of the estrous cycle, many induced corpora lutea were beginning to regress. This is a finding not too dissimilar from the effects of oxytocin injected early in the estrual cycle (Simmons and Hansel, 6). In light of recent findings on factors affecting the life of the bovine corpus luteum, this effect may be interpreted as an indication of an LH deficiency which is responsible for failure of the corpus luteum to persist the usual length of time (Simmons and Hansel, 6). These findings would tend to discourage synchronization techniques which involve induction of ovulation during a period of continued progestogen treatment or during persistence of progestogen levels.

General Discussion

The increased follicular activity and cervical mucus smear ratings suggest very little alteration of the FSH (follicle stimulating) levels in treated heifers. According to one concept of stimuli needed for estrogen production, it could also be speculated that there must be some LH influence on the ovary as well as FSH influence. In recent years, several workers have concluded that two hypothalamic centers exist for release of pituitary LH (Barraclough and Gorski, 2). One center is postulated to control tonic release of small amounts of LH and the other is regarded as controlling the cyclic release of large amounts of LH as needed for ovulation. Thus, one interpretation of the mechanism of oral progestogen inhibition is that only the cyclic release of pituitary LH in the amounts needed for ovulation has been inhibited. This would allow normal FSH levels and levels of tonic LH adequate to produce the large follicles and the apparent resultant estrogenic influence.

---

[2] Chorionic Gonadotropin (Upjohn).

[3] P.L.H. (Armour).

Of the presently known methods of altering the estrous cycle of the cow, the most promising at present appears to involve the inhibition of ovulation. Of the various possible means to inhibit ovulation, the use of progestational steroids appears to be receiving the most attention. Some of the presently used progestogens are very difficult to characterize. The relative potency by a common route varies markedly with species and the potency within a species varies markedly by route of administration. One cannot characterize hormones, he can only describe the effects of certain dosages of hormones by a given route. Thus, it seems necessary to adequately determine the physiologically effective dose by the intended route of administration prior to comparing two hormones or compounds.

Summary and Conclusions

Various methods of altering the estrual cycle of the cow were discussed. Ovulation inhibition was selected for further discussion and data relating to the use of progestogens for this purpose were discussed. The conception rate at the synchronized estrus varied from 26 to 75% in various trials, but was consistently high at the second cycle. This type of variation requires that comparisons of compounds or procedures used be made completely contemporaneous. Ovulation was synchronized in post-partum beef cows placed on treatment prior to the resumption of normal estrual cycles. Additional work is needed on other compounds and with cows that require longer intervals to first post-partum estrual cycle. A single subcutaneous injection of 100 to 500 mg. MAP prevented ovulation for more than 106 days in many heifers. The residual MAP was removed and analyzed at slaughter and as much as 46% remained after 106 days. Follicular activity was greater during the period in which ovulation was inhibited than during the normal estrual cycle. Both the size and incidence of palpable follicles increased following a single injection of MAP. Cervical mucus smears showed typical fern patterns suggesting that the increased follicle size during oral progestogen treatment was associated with increased estrogen production. These findings should be considered in the interpretation of side effects and after effects. The progestogen block of ovulation can be overcome by administration of estrogens in the proper vehicles and route and by various gonadotropins (pituitary luteinizing hormone and Chorionic Gonadotropin). Corpus luteum formation and persistence were not always of normal proportions, however. Such findings tend to discourage synchronization techniques which induce ovulation in cattle during a period of continued progestogen influence. Discussion supports the concept that ovulation inhibition with progestogens was accomplished by inhibition of the mechanism causing cyclic release of pituitary ovulating hormone.

Acknowledgment

The author wishes to acknowledge the fine assistance given by his colleague, LaMont W. Smith, in many of these studies. Thanks are also due J. I. Northam for aid in the statistical analysis and A. W. Neff for aid in chemical analysis of MAP residues from ears of injected heifers.

Literature Cited

(1) Alliston, C. W., Patterson, T. B., and Ulberg, L. C.
1958. Crystallization Patterns of Cervical Mucus as Related to Estrus in Beef Cattle. Jour. Anim. Sci. 17: 322.

(2) Barraclough, C. A., and Gorski, R. A.
1961. Evidence That the Hypothalamus Is Responsible for Androgen-Induced Sterility in The Female Rat. Endocrinology 68: 68.

(3) Foote, W. D., Hauser, E. R. and Casida, L. E.
1960. Influence of Progesterone Treatment on Post-Partum Reproductive Activity in Beef Cattle. Jour. Anim. Sci. 19: 674.

(4) Foote, W. D., and Hunter, J. E.
1964. Post-Partum Intervals of Beef Cows Treated With Progesterone and Estrogen. Jour. Anim. Sci. 23: 517.

(5) Hansel, W.
1959. The Estrous Cycle of The Cow. Reproduction in Domestic Animals, Vol. I, Ed. H. H. Cole and P. T. Cupps. Academic Press.

(6) Simmons, K. R., and Hansel, W.
1964. Nature of The Luteotropic Hormone in The Bovine. Jour. Anim. Sci. 23: 136.

(7) VanBlake, H., Brunner, M. A. and Hansel, W.
1963. Use of 6-Chloro-$<$6-Dehydro-17-Acetoxyprogesterone (CAP) in Estrous Cycle Synchronization of Dairy Cattle. Jour. Dairy Sci. 46: 459.

(8) Wagner, J. F., McAskill, J. W., and Means, T. M.
1963. Synchronization of Estrus in the Bovine. Jour. Anim. Sci. 22: 866. (Abstract)

(9) Zimbelman, R. G.
1963. Determination of the Minimal Effective Dose of 6a(-Methyl-17a(-Acetoxyprogesterone for Control of the Estrual Cycle of Cattle. Jour. Anim. Sci. 22:1051.

(10) Hansel, W.
1964. Personal Communication.

(11) Zimbelman, R. G.
1964. Unpublished data.

Table 1.--Conception rates of heifers after synchronization with MAP

Number of Animals:	Dairy Heifers	Beef Heifers - 1961[b] B1	B2	B3
Treated	46	36	35	24
Synchronized	45	35	35	19
Untreated	8	-	-	12
Conception Rates (Percent):				
S1[a]	58%	60%	26%	37%
S2	62%	87%	85%	83%
S1 + S2	85%	94%	89%	92%
S26	78%	67%	77%	75%
U1	75%	-	-	67%

[a] S1 = First service of treated (synchronized) animals.
S2 = Second service of treated animals.
S1 + S2 = Treated animals conceiving with two services.
S26 = Treated animals conceiving within 26 days after last feeding.
U1 = First service of untreated (control) animals.

[b] Data on 3 beef heifer trials (B1, B2, B3) combined in table 2.

Table 2.--Conception rates of heifers and cows after synchronization with MAP

Number of Animals:	Dairy Heifers	Dairy Heifers	Beef Cows[b] 1962	1963	Total
Treated	46	95	22	51	214
Synchronized	45	89	20	42	196
Untreated	8	12	18	17	55
Conception Rates (Percent):					
S1[a]	58%	42%	75%	39%	47%
S2	62%	85%	50%	81%	78%
S1 + S2	85%	91%	86%	88%	89%
S26	78%	76%	86%	78%	77%
U1	75%	67%	78%	59%	69%

[a] See table 1 for explanation of abbreviations.

[b] All beef cows were bred by natural service.

Table 3.--Design of studies to determine influence of MAP on post-partum reproductive performance of beef cows

	Controls		Treated 180 mg. MAP			Year total
Days PP[a]	9-15	16-22	9-15	16-22	16-22	
Days Fed	17	17	17	10	17	-
			Number of Cows			
Year						
1962	9	9	12	0	10	40
1963	17	0	17	17	17	68
Total	26	9	29	17	27	
Pooled Total	35		73			108

[a] Days PP = Days from calving to first grain feeding.

Table 4.--Average intervals from calving as influenced by MAP

	Controls		Treated 180 mg. MAP		
Days PP	9-15	16-22	9-15	16-22	16-22
Days Fed	17	17	17	10	17
Last Feeding[a]	27.7	35.2	27.9	27.0	35.2
	Days Post-Partum to: First Post-Treatment Ovulation				
Year					
1962	36.6	43.6	32.5	-	39.9
1963	35.9	-	32.6	32.8	40.0
	Conception Rates (Percent)				
1962	39.1	59.7	43.9	-	41.6
1963	50.3	-	51.3	51.5	52.5

[a] The average interval from calving to last feeding was obtained for the two years.

Table 5.--Average intervals and variation to first post-treatment ovulation

	Controls			Treated 180 mg. MAP			
Days PP	9-15	16-22	Pooled	9-15	16-22	16-22	Pooled
Days Fed	17	17	-	17	10	17	-
	Days From Last Feeding: Mean						
Year							
1962	8.0	8.4	8.2	4.2	-	4.5	4.3[a]
1963	8.9	-	8.9	4.9	5.8	5.1	5.3[b]
	Standard Deviation						
1962	6.3	9.5	7.8	1.7[c]	-	3.5[c]	2.6[d]
1963	7.8	-	7.8	4.7	6.5	5.1	5.4[b]

[a] Differs from pooled control ($.06>P>.05$).
[b] Differs from pooled controls ($P<.05$).
[c] Differs from respective control ($P<.01$).
[d] Differs from pooled controls ($P<.001$).

Table 6.--Distribution of first post-treatment ovulation after last MAP feeding

Days After Last Feeding	Percent of Cows	
	Controls	Treated
0 to 1	17%	3%
2 to 6	34%	79%
7 to 20	43%[a]	14%
21 or >	6%	4%
N	35	73

Chi-Square Value: 22.75 ($P<.005$)

[a] Of 15 cows in this group, 7 had apparently ovulated during the feeding period.

Table 7.--Conception rates of post-partum cows

	Controls	Treated 180 mg. MAP			
Days PP	-	9-15	16-22	16-22	Pooled
Days Fed	17	17	10	17	-
		First Cycle			
Year					
1962	78%	58%	-	90%	73%
1963	59%	35%	29%	53%	39%
		Second Cycle			
1962	50%	40%	-	100%	50%
1963	57%	82%	75%	88%	81%
		Two Cycles			
1962	89%	75%	-	100%	86%
1963	82%	88%	82%	94%	88%
		By Day 26 ALF[a]			
1962	72%	75%	-	100%	86%
1963	71%	76%	71%	88%	78%

[a] Percentage of group pregnant by 26 days after last feeding of MAP.

Table 8.--Distribution of interval from calving to conception

	Cumulative Percent of Cows Conceiving[a]		
Days Post-Partum	Controls	Treated	All Cows
27 to 39	46%	32%	36%
40 to 49	69%	60%	63%
50 to 59	74%	81%	79%
60 or >	100%	99%	99%
N	35	73	108

Chi-Square: 5.67, ($P<.10$) for comparison of controls and treated.

[a] Based on cows conceiving with 4 or less services.

Table 9.--Intervals between calvings and gestation length

	Controls	Treated	All Cows
Number of Cows	18	20	38
Average Intervals:			
Calving to Conception	49.4	44.5	46.8
Calving to Calving[a]	332.9	325.9	329.2
Gestation length	283.5	281.4	282.4

[a] The number of days from date calved in 1962 to date calved in 1963.

Table 10.--Recovery of residual MAP from ears of injected heifers

		MAP Recovered			Mg. MAP Absorbed	
	Mg. MAP	Range	Mean			
Trial	Injected	(Mg.)	Mg.	%	Total	Daily
A	100	0 to 43	21	21	79	0.62[a]
	500	18 to 212	76	15	424	3.37
B	50	4 to 36	21	42	29	0.26[b]
	100	21 to 69	46	46	54	0.48

[a] The test period was 126 days for trial A.

[b] Five heifers were killed at each of 106, 110, 113, and 117 days from injection, averaging 111.5 days for the 20 animals.

Table 11.--Summary of ovarian activity following single MAP injections

		Rectal Palpation				
		Overall Incidence[a]			Slaughter Findings	
MAP Injected	No. Heifers	Corpora Lutea	Follicles	Follicle[b] Size	New C.L.	Old C.L.
-	13	71%	37%	14.0	100%	100%
100	13	7%	84%	16.6	64%	18%
500	13	3%	81%	14.8	8%	0%
(N)[c]		(230)	(230)	(154)		
-	20	78%	38%	14.0	100%	100%
50	20	54%	61%	14.9	100%	95%
100	20	32%	77%	16.3	65%	65%
(N)		(510)	(510)	(298)		

[a] Percent of rectal palpations at which a corpus luteum or follicle was detectable.

[b] The average size (mm.) of follicles as estimated on rectal palpation.

[c] Indicates the number of rectal palpations performed or the number of follicles measured.

Table 12.--Ovarian weights of heifers treated with MAP

			Ovarian Weight		Follicular Fluid Weight[b]	
Trial	MAP Injected	No. Heifers	Total Intact (Grams)	After Slicing[a] (Grams)	Largest Follicle (Grams)	Total (Grams)
A	-	13	11.34	9.93[c d]	-	1.41
	100	11	14.79	10.85[c]	-	3.94[d]
	500	13	11.77	7.39[d]	-	4.38[d]
		D[e]	3.49	2.81		2.10
B	-	20	13.30	11.34	1.08	1.96
	50	20	14.38	12.30	1.21	2.07
	100	20	14.71	11.74	1.83	2.97
		D[e]	2.63	2.17	.97	1.30

[a] The ovary was sliced at 5 mm. intervals and fluid was blotted.

[b] The follicular fluid weight represents the difference between total ovarian weight and weight after puncture of the largest follicle or after slicing at 5 mm. intervals.

[c] [d] Mean values bearing the same superscript are not significantly different ($P < .05$).

[e] D = Difference required for statistical significance ($P < .05$).

Table 13.--Cervical mucus smear ratings of dairy heifers receiving oral progestogen for 8 or more days

	During progestogen treatment	
Percent rated as:[a]	Mature corpus luteum	Corpus luteum regressed[b]
1 or 2	83%	28%
3 or 4	17%	47%
5 or 6	0%	25%
N	41	128

Chi-Square 39.78 ($P < .005$)

[a] See text for description of rating procedure.

[b] Regression defined as no palpable corpus luteum or estimated size decreased by 4 mm. or more.

HORMONAL CONTROL OF REPRODUCTIVE ACTIVITY IN THE EWE

J. F. Wagner
Eli Lilly and Company, Greenfield, Indiana

Introduction

There are many advantages which result from synchronizing the estrous period in a ewe flock. In situations where the ewes are not hand bred to the ram, the synchronization of estrus makes the application of artificial insemination (AI) techniques more efficient. Detection of estrus usually is more reliable in a synchronized flock. Only 3 to 4 days are needed to detect estrus in 90 percent of a synchronized flock, whereas 16 to 18 days are normally required. This allows the AI technician to plan the number of breedings and have semen available. When hormones are given in the feed, an opportunity is presented for improving the nutritional status of the flock during the breeding period. Synchronization coupled with AI will increase the genetic uniformity of the lamb crop as well as uniformity of age. Attention and labor required at lambing could be easily scheduled and concentrated within a short period of time.

Progesterone injections have been used for controlling estrus and ovulation in cattle, sheep, and swine. The development of potent orally active progestins is responsible for much of the recent interest in estrous cycle control. The first progestin shown to have significant oral activity in the ruminant was 6-methyl-17 acetoxyprogesterone (MAP) (8, 9, 11).[1] Wagner and Bush (12) reported that 6-chloro-Δ^6-17 acetoxyprogesterone (CAP) when given orally was effective in inhibiting estrus and ovulation in the ewe. The first portion of this paper presents data concerning the effects of CAP on estrus, ovulation, and fertility in cycling ewes.

Many breeds of sheep show a period of reproductive quiescence during the late winter, spring, and summer months. During the fall and early winter the ewe has a regular estrous cycle every 16 to 17 days. Therefore, during 7 months of the year many ewes are producing nothing more than wool and milk for their lambs. This period of reproductive inactivity is characterized by a failure of Graafian follicle maturation, estrus, and ovulation. Consequently, any attempt to hormonally induce reproductive activity in the anestrous ewe requires an exogenous follicle stimulating hormone.

The use of pregnant mare serum (PMS) as an inexpensive source of follicle stimulating activity has made possible the practical application of physiological principles for the induction of reproductive activity in the anestrous ewe. Many approaches have been used to induce reproductive activity with PMS alone. Cole and Miller (4) introduced the method of two injections of PMS spaced at a 16-day interval. Cole *et al.* (3), Dutt (5), and Robinson (10) preceded the PMS injection with a series of progesterone injections. Gordon (6) and Allen and Lamming (1) have attempted with little success to induce fertile estrus in the lactating ewe. Orally active progestins have increased the efficiency of hormone application. Wagner and Bush (12) used an orally active progestin in the hormone sequence for the induction of estrus in the ewe. Anderson *et al.* (2) have summarized the recent literature concerning hormonal induction of reproductive activity in the anestrous ewe.

The second portion of this paper presents studies which were designed to investigate the interaction of three hormones, 6-chloro-Δ^6-17 acetoxyprogesterone (CAP, Na-estradiol (NE), and PMS and other variables such as postpartum interval and lactation status affecting the induction of estrus and ovulation in the anestrous ewe.

Experimental Methods

The data presented were compiled during the past 4 years at South Dakota State University and the Eli Lilly Agricultural Research facilities. The majority of the sheep used in these studies were black-faced and white-faced crossbreds; the two breeds were equally represented in all treatment groups. All ewes treated ranged from 2 to 4 years of age and had produced lambs in the spring prior to treatment.

In tables 1 through 4 data have been compiled from four experiments over 3 successive years on the effects of CAP in the cycling ewe. Dosages of CAP ranging from 0.25 mg. to 75.0 mg. per ewe per day for 16 days were given in the feed (table 1). The CAP was dissolved in one liter of ethanol or propylene glycol and added to 15 lb. of alfalfa grits which was used as a premix containing 0.1 gm. of CAP per pound. Sufficient premix was added to a concentrate ration containing a minimum of 60 percent ground corn so that the daily dosage of CAP could be supplied by 1.0 lb. of finished feed.

The ewes were fed in groups ranging from 12 to 106 ewes. When in drylot, the 1.0 lb. of hormone feed was given in the morning separate from and prior to the daily maintenance ration. When on pasture, the ewes were placed in the drylot overnight and given the hormone feed before they were returned to pasture in the morning.

---

[1] Underscored numbers in parentheses refer to items in the Literature Cited, p. .

Results in the CAP-fed ewes were compared with those obtained in ewes injected with 10 mg. of progesterone in 1 ml. of corn oil subcutaneously daily for 16 days. These progesterone-treated control ewes were handled the same as the treated ewes in all other respects.

A minimum of two estrous periods (one cycle) was observed in all ewes before they were placed on progestin treatment. All stages of the estrous cycle were equally represented in each treatment group. The ewes were identified with ear tags and paint brands.

Prior to and during treatment vasectomized rams were used to check each ewe for estrus. Two to three ewes at a time were placed with a single ram once per day in small pen and estrus was observed directly. Following treatment, the same procedure for observing estrus was employed using fertile rams three times a day. In an attempt to reduce the differences in conception rates due to variation in ram fertility, most ewes were bred to at least two different rams per estrous period. Twice-daily heat checks were started 6 days after treatment and continued for 25 to 45 days. The average interval from the end of treatment to the onset of estrus (AIE) was recorded and the standard deviation of these intervals was used as a measure of the degree of synchronization.

Conception dates were verified at lambing using gestation periods of 146 days for the black-faced and 148 days for the white-faced crossbreds (table 4).

Twelve ewes (table 2) were divided into 2 groups of 6 each, with one group receiving 4.0 mg. of CAP per ewe per day for 10 days in the feed and the other group receiving 4.0 mg. of CAP plus 4.0 mg. of ethynylestradiol-3 methyl ether (3ME) for 10 days. Treatment was started on day 8 of the estrous cycle. The ewes were laparotomized the last day of treatment (day 18 of cycle) and the size and number of follicles recorded.

Ovulation data were observed in 12 other ewes receiving in the feed as described above various combinations of CAP alone and with 3ME (table 3). The ewes were laparotomized 8.5 days after the end of steroid treatment and the size and number of follicles and corpora lutea were recorded.

Tables 5 through 13 include data accumulated over a 2-year period on the effects of CAP, pregnant mare serum (PMS), and Na-estradiol (NE) on the induction of estrus and ovulation in the anestrous ewe. The ewes in these studies were the same animals used for synchronization and all had lambed in the spring prior to treatment.

One-half of all the ewes received 0.5 mg. per ewe per day of CAP for 16 days, and the other half received 1.0 mg. per ewe per day. The ewes received the hormone in the feed in the manner as described above in groups 12 to 24. All ewes were in drylot during and 25 days following treatment. Ewes were maintained on an alfalfa-hay plus mineral ration or a complete breeding ration.

Ewes were checked for heat using the procedures previously described. Beginning on the day of PMS injection, the ewes were checked for heat 3 times a day for 4 to 5 days. Twenty rams were available to breed each 100 ewes placed on treatment at a given time.

All ewes received 1,000 I.U. PMS[2] injected subcutaneously in the inguinal region. In the first experiment the PMS was given 12, 24, or 36 hours after the last CAP feeding, and a second PMS was given 16.5 days after the first PMS to ewes that did not show estrus following the first PMS (table 5.) Half of these ewes were lactating and half were dry.

In the second study, 96 ewes were divided into four groups, one-half of the ewes receiving 0.5 mg. CAP per ewe per day and the other half 1.0 mg. CAP per ewe per day for 16 days. These were further subdivided into a 2 x 2 factorial with half of the ewes receiving 500 µg. of Na-estradiol injected subcutaneously and the other half receiving no Na-estradiol (table 6). All of these ewes and their lambs weaned at least 2 weeks prior to treatment.

In the third study (tables 7, 8, 9, and 10) all ewes were lactating at the time of breeding, with intervals between spring lambing and spring breeding ranging from 30 to 79 days. The first PMS was given 36 hours after the last CAP feeding and the second PMS was given 16.5 days after the first PMS. The second PMS was given under three different conditions: (A) All ewes received 1,000 I.U. of PMS 36 hours after the last CAP feed, and ewes showing estrus were bred to fertile rams. Ewes not showing estrus were given a second 1,000 I.U. of PMS 16.5 days after the first PMS. (B) All ewes received one injection of 1,000 I.U. of PMS 36 hours after the last CAP feeding, and another 18 days after the last CAP feeding. Ewes showing estrus were bred to fertile rams. (C) All ewes received two injections of PMS as described in (B); however, vasectomized rams were used after the first PMS injection and fertile rams after the second PMS (table 12). None of the ewes received CAP between the first and second PMS injections.

The ewes were divided into five groups depending upon their interval from lambing to breeding (tables 7, 8, 9, and 10). Ten-day periods were arbitrarily selected for each interval group. Any ewe showing an estrous period following both the first and second PMS would appear twice in these tables in either the "ewes conceived" or "ewes open" column.

Thirty-six ewes were selected from the first study for laparotomy 5 days after the first PMS. Thirteen of these ewes had shown estrus within 5 days following the first PMS and 23 had not shown estrus. Corpora lutea of at least 6 mm. in diameter and red in color were designated as ovulation sites attributable to the PMS injection.

---

[2] Lyophilized PMS - Ferring AB, Malmo, Sweden

In the first experiment 84 lactating ewes with post-partum intervals of 30 to 69 days at the time of the first PMS injection were compared with 85 nonlactating ewes with the same post-partum intervals (table 13). The nonlactating ewes were weaned from their lambs at least 2 weeks prior to the initiation of treatment. However, some ewes had lost their lambs following lambing and had been dry for 2 to 8 weeks.

Data from experiments 1, 2, and 3 were compiled so as to compare results between years A and B (table 14). Year A represents experiment 2 and year B experiments 1 and 3. Only estrus and conception data from the first PMS injection are included in this comparison.

Results and Discussion

Cycling Ewes. Data from four experiments on the incidence of estrus and synchronization are reviewed over a dosage range of 0.25 mg. to 75.0 mg. per ewe per day of CAP (table 1). The effective dosage range for estrous response appears to be 1.0 to 6.0 mg. per ewe per day of CAP for a period of 16 days. The AIE increased as the CAP dosage increased from 1.0 mg. to 6.0 mg. Synchronization as measured by the standard deviation of the intervals from end of CAP treatment to estrus clearly indicates 1.0 mg. per ewe per day CAP as the optimum dosage level. Dosages of 0.25 and 0.50 mg. per ewe per day CAP resulted in a significant number of ewes showing estrus throughout the 16-day treatment period (table 1). The AIE of the positive control ewes receiving 10 mg. of progesterone in oil subcutaneously daily for 16 days was not significantly different from the 1.0 mg. CAP dosage (3.1 days vs. 3.2 days, respectively).

The development of follicles on the ovary during and after CAP treatments was studied, and the results are shown in tables 2 and 3. The 4.0 mg. dosage of CAP alone did not completely inhibit follicular growth, whereas with the addition of 4.0 mg. per ewe per day of 3ME, complete inhibition of follicular development during treatment was noted (table 2). Eight and one-half days after a 2.0 mg. dosage of CAP, 11 of 12 ewes had ovulated and no 3ME effect on ovulation was seen (table 3).

Conception data for the effective dosage range of estrous response (1.0 - 6.0 mg. of CAP) appears in table 4. At the first synchronized estrus, conception rates ranged from 23 percent to 38 percent of the total ewes treated. The positive controls had the highest first service conception rate (64 percent); the 1.0 mg. and 2.0 mg. CAP dosages had higher conception rates than CAP at 4 mg. and 6 mg. per ewe per day. Following two services, conception rates ranged from 77 percent to 86 percent over all treatments. However, no significant differences in conception rates after two services were observed between treatments.

Anestrous Ewes. The effects of three time intervals (12, 24, and 36 hours) between the last CAP treatment and a 1,000-I.U. PMS injection (subcutaneous) are shown in table 5. The percentage of ewes in estrus was significantly higher (70 percent vs. 43 percent - $P < 0.05$) than the percentage in heat in the 12- and 24-hour CAP-PMS treatments. Estrous responses following a second PMS injection 16.5 days after the initial PMS were approximately the same for each CAP-PMS interval regime (range 70 percent to 75 percent). The average AIE was significantly shortened ($P < 0.05$) as the CAP-PMS intervals were increased. The AIE after the second PMS was not influenced by the CAP-PMS intervals. Estrous synchronization was decreased (standard deviation increased) following the second PMS injection ($P < 0.05$) when compared to the initial estrous synchronization.

The use of NE (500 μg) injected subcutaneously 36 hours following PMS (1,000 I.U.) increased the percentage of ewes showing estrus (table 6). However, those ewes receiving the NE had a significantly lower conception rate ($P < 0.05$) when compared to those receiving only the CAP-PMS treatment.

The interval from parturition to the CAP-PMS induced estrus was studied relative to its effects upon the induction of estrus and conception. The interval from spring lambing to the hormone-induced estrus following the first PMS ranged from 30 to 69 days (table 7). The post-partum interval (PPI) had no significant effect upon the induction of estrus. However, the PPI did have a significant effect upon conception. It is shown in table 8 that ewes bred between 50-59 days post partum had the highest conception rate ($\chi^2 = 15.57$).

The average PPI to conception for 107 ewes was 47.4 ± 14.5 days. Eighty-seven of these ewes were allowed to breed naturally after lambing in the fall (October). The PPI to conception in the fall was 48.5 ± 14.9 days. It is apparent that similar time relationships governing the ability of the ewe to conceive while lactating are in effect during both the spring and fall months. A covariance of PPI to conception was calculated between spring and fall; no correlation was noted ($r = -.13$).

Ewes showing estrus following the second PMS injection had a higher conception rate when compared to ewes bred following the first PMS treatment (1st PMS 56.9 percent vs. 2d PMS 72.0 percent and 66.7 percent - $P < 0.05$, tables 8, 9, and 10). This higher conception rate following the second PMS could be due to two factors: (1) the average PPI to conception was 16.5 days longer, and

(2) the second PMS was given in the presence of a regressing corpus luteum, whereas the first PMS was given following CAP treatment.

The shortest PPI at the time of the second PMS injection was 42 days, which could account for PPI not affecting the conception rate following the second PMS (tables 9 and 10) ($\chi^2 = 0.7$ and 0.3, respectively). Considering PPI independently of first or second PMS injection it would appear that 40 to 60 days following spring lambing are necessary for optimal conception to a hormonally induced estrus.

Gordon, 1963, reported that lactating ewes had a low conception rate following a progestin-PMS treatment, but following a second progestin-PMS regime, the conception rate was significantly improved. However, Gordon's studies as well as the ones described in this report do not separate the effects on conception of post-partum interval from the effects on conception of the second PMS.

It was assumed that the second PMS was given in the presence of a regressing corpus luteum and it should be noted that 35 of 36 ewes ovulated following a CAP-PMS treatment (table 11). Twenty-three of these 36 ewes had not shown estrus following PMS; however, 22 ovulated within 5 days after the PMS injection.

When the second PMS was given to a group of ewes which conceived following the first PMS, no differences in conception rate could be shown when compared to ewes which received a single PMS injection (Treatment A vs. B, table 12). Fifty percent of 46 ewes conceived following an initial injection of PMS; 62.9 percent of 27 ewes which received two PMS injections conceived following the first PMS. Of the 40 ewes not bred following the first PMS injection, 24 (60 percent) came into estrus following the second PMS injection and 66.6 percent of these lambed (table 12). Compared to the incidence of estrus following the first PMS, this was a reduction of 12.5 percent.

The effect of lactation upon the hormonal induction of estrus and conception in the ewe is shown in table 13. In this study the lactating ewes had a significantly higher incidence of estrus and rate of conception ($P < 0.05$) when compared to the nonlactating ewes.

The ewes treated with 0.5 mg. of CAP per day for 16 days followed 24 hours later by PMS showed a significantly higher incidence of estrus in year A; in year B there was no difference between 0.5 mg. and 1.0 mg. of CAP (table 14). Of those ewes which showed heat, no difference in conception rates could be seen in year A; in year B the 1.0 mg. CAP dose was significantly higher ($P < 0.05$).

Summary

In the fall months, ewes in which at least two estrous periods were observed were fed various levels of CAP for a 16-day period. Upon withdrawal of CAP from the feed, the incidence of estrus, incidence and rate of ovulation, and percentage of conception were recorded. Dosages of CAP ranging from 0.25 mg. to 75.0 mg. per ewe per day were administered in the feed. The minimal dosage of CAP for complete inhibition of estrus and ovulation was 1.0 mg. per ewe per day. Eighty percent of the ewes treated with 1.0 mg. CAP came into a synchronized estrus following treatment. Ninety-three percent of the positive controls (10 mg. per ewe per day of progesterone injected daily for 16 days) showed estrus following treatment. The conception rates of the first two services following treatment in the 1.0 mg. dosage group and the positive control group were 86 and 84 percent, respectively. The conception rate at the first service subsequent to treatment was higher in the positive controls.

Lactating and anestrous ewes were treated with CAP for 16 days followed by an injection of 1,000 I.U. of PMS. Na-estradiol was added as supplemental estrogen to some ewes 36 hours after PMS. In addition, a second PMS injection was used in various studies 16.5 days after the first PMS treatment. The optimum interval from the last CAP treatment to the first PMS injection was 36 hours. The use of 500 μg. of NE to supplement the estrogen from the PMS-induced follicle reduced the conception rate significantly. The interval between parturition and breeding had a significant effect on conception, but no effect upon the induction of estrus. Forty to 60 days after lambing were necessary for an optimal conception rate. Ovulation occurred following CAP-PMS treatment in 35 of 36 ewes. Twenty-three of these ewes had not shown estrus following PMS, but 22 had ovulated. A second PMS 16.5 days after the first PMS was therefore employed to induce another estrus in the presence of a regressing corpus luteum resulting from the first PMS treatment. In those ewes showing estrus the conception rate was significantly higher after the second PMS when compared to the conception rate following the first PMS. The second PMS did not affect conception rate following the first PMS. In one study lactating ewes appeared to have a higher percentage of ewes in estrus and a higher lambing percentage when compared to nonlactating ewes. The optimal dosage of CAP in the hormone sequence ranged between 0.5 mg. and 1.0 mg. per ewe per day.

Literature Cited

(1) Allen, D. M. and Lamming, G. E.
1960. The Induction of Breeding Activity in Lactating Ewes during Anestrous. Jour. Reprod. and Fertil. 1: 213.

(2) Anderson, L. L., Schultz, J. R., and Melampy, R. M.
1964. Gonadotropins, Their Chemical and Biological Properties and Secretory Control. Ed. H. H. Cole. Pharmacological Control of Ovarian Function and Estrus in Domestic Animals. Sixth Animal Reprod. Symposium, Proc. p. 170. U. H. Freeman and Co.

(3) Cole, H. H., Hart, G. H., and Miller, R. F.
1945. Studies on the Hormonal Control of Estrous Phenomena in the Anestrous Ewe. Endocrinology 36: 370.

(4) Cole, H. H. and Miller, R. F.
1933. Artificial Induction of Ovulation and Oestrum in the Ewe During Anestrous. Amer. Jour. Physiol. 104: 165.

(5) Dutt, R. H.
1953. Induction of Estrus and Ovulation in Anestrual Ewes by the Use of Progesterone and PMS. Jour. Anim. Sci. 12: 515.

(6) Gordon, I.
1958. The Use of Progesterone and Serum Gonadotropin (PMS) in the Control of Fertility in Sheep. II. Studies in Extra-Seasonal Production of Lambs. Jour. Agr. Sci. 50: 152.

(7) Gordon, I.
1963. The Induction of Pregnancy in the Anestrous Ewe by Hormonal Therapy. III. The Use of Repeated Progesterone-Pregnant Mare's Serum Therapy. Jour. Agr. Sci. 60: 67.

(8) Hansel, W. and Malven, P. V.
1960. Estrous Cycle Regulation in Beef Cattle by Orally Active Progestational Agents. Jour. Anim. Sci. 19: 1324 (Abstr.).

(9) Nellor, J. E. and Ahrenhold, J. E.
1960. The Influence of Oral Administration of 6-Methyl-17-Acetoxyprogesterone on Follicular Development and Estrous Behavior in Heifers. The Physiologist 3: 3 (Abstr.).

(10) Robinson, T. J.
1955. Endocrine Relationships in the Induction of Oestrus and Ovulation in the Anestrous Ewe. Jour. Agr. Sci. 46: 37.

(11) Simpson, E. C. and Dutt, R. H.
1960. Synchronizing Estrus in Ewes by Feeding an Orally-Active Progestin. Univ. Ky. Agr. Expt. Sta. Ann. Rpt. 73: 34.

(12) Wagner, J. F. and Bush, L. F.
1961. Orally Active Progestin in the Hormonal Control of Reproductive Activity in the Cycling and Estrous Ewe. Jour. Anim. Sci. 20: 980 (Abstr.).

Table 1.--CAP levels for the synchronization of estrus in the ewe, summary of 4 experiments

Treatment[a] CAP mg/ewe/day	No. of ewes	Ewes[b] in estrus during treat. %	Ewes[c] in estrus following treat. %	AIE[d] Days**	± St. dev.**
0.25	20	80	0	-	-
0.50	72	8	25	4.2	1.5
1.0	279	0	80	3.2	0.8
2.0	22	0	73	4.6	0.9
4.0	22	0	78	5.9	2.6
6.0	22	0	95	7.4	3.1
25.0	9	0	11	12.0	-
50.0	9	0	0	-	-
75.0	9	0	0	-	-
Progesterone[e]	45	0	93	3.1	0.5

[a] CAP given in 1.0 lb. of concentrate daily for 16 days.

[b] Ewes showing estrus within 24 hours after the initiation of treatment are not included.

[c] This percentage includes all ewes in estrus between 2.5 and 9.0 days after the withdrawal of CAP except for the 25.0 mg. CAP treatment.

[d] AIE = Average interval from end of CAP treatment to onset of estrus.

[e] Ten mg. of progesterone in oil per ewe were injected subcutaneously daily for 16 days.

** With the exception of the 0.5 mg. CAP dose, these values increase significantly ($P < 0.01$) as the CAP dose increases, (CAP dose vs. interval - $r = 0.9$).

Table 2.--The effect of CAP and 3ME upon follicular development in the ewe

Treatment CAP mg/day	Treatment 3ME mg/day	No. of ewes	Day of cycle treat. started	Day of cycle treat ended[a]	Follicles Av. no.	Follicles Size mm.[b]
4	0	6	8	18	3.2	5.8
4	4	6	8	18	0	0

[a] Ewes laparotomized on last day of treatment; follicles and corpora lutea measured.

[b] No follicles 2 mm. or less were recorded.

Table 3.--Ovulation in the cycling ewe following steroid treatment

Treatment CAP mg/day	Treatment 3ME mg/day	No. of ewes	Ovarian response following treatment[a] Average follicle size mm.	Average CL size mm.	No. of ewes ovulated
2	0	4	8	8	4
2	1	4	7	9	3
2	4	4	6	7	4

[a] Laparotomy performed 8.5 days after end of treatment.

Table 4.--The incidence of estrus and the percentage of ewes conceiving subsequent to CAP treatment

Treatment[a] CAP mg/ewe/day	No. of ewes treated	First estrus follow. treat. %[b]	Ewes lambed %[b]	Second estrus follow. treat. %[b]	Ewes lambed %[b]	Ewes lambed to first + second estrus %[b]
1	279	80	38*	59	48	86
2	22	73	36	59	45	82
4	22	77	27	64	54	82
6	22	95	23	73	54	77
Progesterone[c]	45	93	64*	36	20	84

[a] CAP fed in 1.0 lb. of concentrate per ewe for 16 days.

[b] Percentages based on total number of ewes treated.

[c] Ten mg. of progesterone in oil per ewe injected subcutaneously daily for 16 days.

* Significant ($P < 0.05$) from 4 and 6 mg. CAP treatment groups.

Table 5.--Effect of CAP-PMS interval on induction of estrus

Int. between end of CAP treat.[a] and PMS inject. hr.	No. of ewes	Ewes in estrus: First serv. %	Second[b] serv. %	First + Second[b] serv. %	AIE[c] First PMS Days ± S.D.		AIE[c] Second PMS Days ± S.D.	
12	59	47	71	88	2.2	0.2	2.7	1.0
24	60	38	75	85	1.8	0.2	2.8	1.0
36	60	70	70	92	1.6	0.3	2.8	0.7

[a] All ewes received 0.5 or 1.0 mg/ewe/day of CAP administered in 1.0 lb. of ground corn for 16 days.

[b] All ewes not showing estrus following the first PMS injection (1,000 I.U.) received a second PMS injection 16.5 days after the initial PMS.

[c] AIE = Average interval from PMS to the onset of estrus.

Table 6.--The effect of CAP-PMS with and without Na-estradiol upon estrus and conception

Treatment[a] CAP mg/ewe/day	Na-estradiol µg/ewe	No. of ewes	Ewes in estrus %	Ewes lambed: Of total treated %	Ewes lambed: Of ewes in estrus %
0.5	-	24	83	42	50
1.0	-	24	33	17	50
Total	-	48	58	30	50
0.5	500	24	92	17	18
1.0	500	24	100	21	21
Total	500	48	96	19	20

[a] All ewes received CAP for 16 days followed by 1,000 I.U. of PMS 24 hours after the last CAP feeding.

Table 7.--Relationship of post-partum interval to the hormonal induction of estrus in the lactating ewes

PPI[a] days	Ewes in estrus No.	Ewes not in estrus No.	Total ewes No.	Total ewes in estrus %
30-39	67	33	100	67.0
40-49	15	1	16	93.8
50-59	42	18	60	70.0
60-69	15	5	20	75.0
Total	139	57	196	71.0

$\chi^2 = 5.1$

[a] PPI = Post-partum interval to the hormone-induced estrus. Ewes received CAP for 16 days followed by two 1,000-I.U. injections of PMS 36 hours and 18 days after the last CAP feeding.

Table 8.--Relationship of conception and post-partum intervals of lactating ewes to the first PMS injection

PPI[a] days	Ewes conceiving No.	Ewes open No.	Total ewes No.	Total ewes conceiving %
30-39	17	27	44	38.6
40-49	8	5	13	61.5
50-59	27	6	33	81.8
60-69	6	6	12	50.0
Total	58	44	102	56.9

$\chi^2 = 15.6$

[a] PPI = Post-partum interval to the hormone-induced estrus. The ewes received CAP for 16 days followed by 1,000-I.U. injections of PMS 36 hours after the last CAP feeding.

Table 9.--Relationship of conception and post-partum interval of lactating ewes receiving a second PMS injection[a]

PPI[b] days	Ewes conceiving No.	Ewes open No.	Total ewes No.	Total ewes conceiving %
50-59	12	6	18	66.6
60-69	5	2	7	71.4
70-79	9	2	11	81.8
Total	26	10	36	72.0

$\chi^2 = 0.7$

[a] The second PMS was given under two experimental conditions:
(1) to ewes which did not show estrus following the first PMS
(2) to ewes which did show estrus following the first PMS and were bred to fertile rams after both first and second PMS.

[b] PPI = Post-partum interval = interval from lambing to estrus following second PMS injection.

Table 10.--Relationship of conception and post-partum interval of lactating ewes receiving a second PMS injection[a]

PPI[b] days	Ewes conceiving No.	Ewes open No.	Total ewes No.	Total ewes conceiving %
40-49	3	2	5	60.0
50-59	5	2	7	71.4
60-69	3	1	4	75.0
70-79	5	3	8	62.5
Total	16	8	24	66.7

$\chi^2 = 0.3$

[a] None of the ewes were bred following the first PMS; therefore, all ewes were nonpregnant at the time of the second PMS injection.

[b] PPI = Post-partum interval = interval from lambing to estrus following second PMS injection.

Table 11.--The effect of CAP-PMS treatment upon ovulation in anestrous ewes[a]

Ewes showing:	Total ewes No.	Ewes ovulated No.	Total ovulations No.	Average No. of ovulations	Range
Estrus[b]	13	13	52	4.0	1-6
No estrus[b]	23	22	58	2.6	0-5
Total	36	35	110	3.1	0-6

[a] All ewes received 0.5 or 1.0 mg. of CAP per day for 16 days followed by 1,000 I.U. of PMS 12, 24, or 36 hours later.

[b] Where possible, ewes were selected for laparotomy 5 days after the first PMS on the basis of one ewe showing estrus and two not showing estrus.

Table 12.--The effect of the second PMS upon conception following the first PMS injection

		1st PMS			2d PMS		
Treatment[a]	No. of ewes	Ewes in heat No.	Ewes lambed No.	%	Ewes in heat No.	Ewes lambed No.	%
A[b]	77	46	23	50.0	19	13	67.4
B[c]	39	27	17	62.9	16	13	81.2
C[d]	40	29	-	-	24	16	66.6

[a] All ewes received either 0.5 or 1.0 mg. of CAP/ewe/day in 1.0 lb. of feed for 16 days.

[b] A = all ewes received 1,000 I.U. of PMS 36 hours after the last CAP feed and those ewes showing estrus were bred to fertile rams. Those ewes not showing estrus were given 1,000 I.U. of PMS 16.5 days after the first PMS.

[c] B = all ewes received two injections of 1,000 I.U. of PMS 36 hours and 18 days after the last CAP feed. Ewes showing heat were bred to fertile rams.

[d] C = all ewes received two injections of 1,000 I.U. of PMS as described in (B); however, vasectomized rams were used after the first PMS injection and fertile rams after the second PMS.

Table 13.--The effect of lactation upon the hormonal induction of estrus and conception in the anestrous ewe[a]

Lactation status	Total ewes No.	Ewes in heat No.	%	Ewes lambed No.	%[b]
Lactating	84	55	65.4	31	56.3
Nonlactating	85	39	45.9	16	41.0

[a] All ewes received 0.5 or 1.0 mg. CAP/ewe/day for 16 days followed by PMS at 12, 24, or 36 hours following the last CAP feed. Second PMS data not included.

[b] The percentage is based on the number of ewes in heat.

Table 14.--The effect of year and level of CAP on the hormonal induction of estrus and conception in the anestrous ewe

Treatment[a]						
Year	CAP mg/ewe/day	Total ewes No.	Ewes in heat No.	%	Ewes conceived No.	%[b]
A	0.5	24	20	83	10	50
	1.0	24	8	33	4	50
B	0.5	137	82	60	36	44
	1.0	148	85	57	51	60

[a] All ewes received CAP in 1.0 lb. of feed for 16 days followed by 1,000 I.U. of PMS per ewe 12, 24, or 36 hours after the last CAP feed. Data of second PMS injection were not included.

[b] The percentage is based on the number of ewes in heat.

DR. DZIUK: I would like to ask Jack Wagner what breed of ewes he is talking about that are anestrous.

DR. WAGNER: Basically, two types, blackface western with a high percentage of Suffolk and Columbia and whiteface westerns with about 50 percent Rambouillet. These were usually equally distributed between all experimental groups. Breeds were not mentioned because we saw no distinction between these two types of breeds, one which tends to show more cycling in the spring.

Incredibly, we saw very few heat periods in the whiteface Rambouillet ewes prior to or following treatment. Nearly all heat periods were inhibited. We checked differences prior to and following treatment.

DR. TERRILL: What was the explanation for the better response in the lactating versus nonlactating ewes?

DR. WAGNER: I have none. This particular response did puzzle us. We were expecting and hoping for equivalent responses indicating it was not lactation but some variable associated with time post-partum.

We have other experiments showing a lower response in the lactating ewe. They cannot be compared directly with these experiments since they were run in different years and different variables were examined, but the lactating ewes did not respond as well as the nonlactating ewes.

DR. CASIDA: I was wondering what arrangements you made to have the two groups comparable with the exception of lactation. How did you select? How did you get your nonlactating ewes?

DR. WAGNER: Post-partum interval was equally distributed in all experimental groups.

DR. CASIDA: But did you deliberately take the lambs away from the nonlactating ewes at birth so the sample used was quite comparable?

DR. WAGNER: The lambs were not removed from the ewes at birth, but as I recall, at 3 or 4 weeks of age. The ewes were placed on treatment 2 weeks later. Post-partum intervals were equally distributed between groups, and half of the ewes had their lambs weaned from them so the post-partum interval would be the same in both groups, the only difference being weaning. These were the only variables that we could control in the experiment. Of course, breeds were equally distributed and weights and sizes of ewes were equally distributed as much as possible between the lactating and nonlactating groups.

DR. CASIDA: Have you any data on birth weights from the ewes that were bred during the otherwise anestrous season for comparison with the normal birth weights of spring-born lambs from the same ewes?

DR. WAGNER: We have the data but we have not looked at them. We probably have around 200 comparisons.

I would say, just from casual observation of procedures in the spring and the fall, that the lambing weights were pretty close together.

I'm afraid we feed our ewes too well to show a real difference. Our lambs are too big really; we have a lot of dystokia. We have triplets that weigh 9 to 10 pounds each and quadruplets that weigh 8 pounds each. In some of these experiments we were running 30 to 40 sets of triplets, and 15 to 20 sets of quadruplets. We were having problems saving all the lambs but not necessarily because they were small.

DR. TERRILL: Are the ewes in drylot or on pasture during the summer?

DR. WAGNER: This was another variable which we investigated; drylot hay vs. drylot grain vs. pasture. There really wasn't much pasture because of a dry fall. Actually, we had two locations with pasture and drylot and then three types of feed: grain (high concentrate, high energy), hay, and pasture. We were expecting to see differences and saw none. About 40 ewes were induced in each of these variables.

DR. NALBANDOV: I would like to ask a question for clarification on one point from you, Dr. Hansel. You mentioned that one of the obstacles to synchronizing heat in swine will be, or is, cystic ovaries.

I have never worked with synchronization of heat in swine but I have done considerable work on feeding progestogens to pigs in corpus luteum studies. We must have gone through, I suppose, nearly 500 pigs, but with proper doses we never get cysts in the ovaries which we have examined at all stages in pregnant and nonpregnant swine. What is the explanation for the discrepancy?

DR. HANSEL: The answer or the key to the answer, of course, is in the phrase "the proper dose."

In making that statement I was, to some extent, quoting Nellor's two papers on this subject where he found a very high incidence of cystic ovaries.

If you will look on the handout (the first experiment on table 4) where swine were fed a progestin (a Searle compound), you will see that half or more of them fed at a level of 0.3 mg. per lb. had cystic follicles. A lesser incidence was noted at 0.5 mg. per lb.

Dose, of course, is a key, and I think, if you get the right dose for every pig, your statement is probably true.

I must ask you, then, Andy, what is your dose level, and how do you control it so accurately? Certainly as one looks through the literature a pretty high incidence of cysts is reported.

DR. NALBANDOV: I will need some help on this. I do not remember the dosages, but I think on 25 mg. of injected progesterone we invariably get cysts, and when we inject 50 mg. we never get cysts. On feeding the progestogen, what is the dose we have used? Dr. Dziuk?

DR. DZIUK: 400 or 500 mg. of MAP in the feed.

DR. NALBANDOV: 400 or 500 mg. results in no cysts.

DR. ZIMBELMAN: One thought has occurred to me which might be important here. I don't know, but I think that at low levels (Dr. First really could speak to this more accurately) the cysts occur during treatment. You are apparently using levels high enough to block this effect. If you have slightly higher levels than these which allow cysts to occur during treatment, then you may get them shortly after withdrawal. If you are feeding higher levels so that you have a longer period after withdrawal until the cysts occur, then you would have a longer period of time in which you found no cysts, and therefore could say cysts do not occur. Perhaps if you waited a longer period of time they might occur. Is this a possibility?

DR. NALBANDOV: No, I think we have covered that in that we have used several intervals. The various feeding experiments for corpus luteum suppression have lasted as long as 3 weeks.

DR. ZIMBELMAN: Yes, but how about the withdrawal periods during which you followed the animals?

DR. NALBANDOV: This we do not have, but my guess is that there would be no cysts even after withdrawal if you use a high enough dose.

DR. HANSEL: There is a tendency, though, where you give higher doses, to have fewer cysts. Everyone seems to get this result. At a lower dose, perhaps a threshold dose, we seem to get more cysts.

My pet explanation for this is that it results from a "leakage" of LH from the pituitary, and that at these threshold levels there is some LH secretion occurring. We think LH is luteotropic, at least in the cow.

DR. MELAMPY: Dr. Hansel, what do you think about climatic or seasonal effects? We seem to observe more cystic ovaries occurring in our untreated animals, the nonexperimental gilts, in the winter months than we do at other seasons. We also find cystic ovaries occurring naturally and normally. In fact we also find cystic ovaries in our so-called normal pregnancies.

I think we have to be careful; maybe some of this is just a normal situation.

Also, what do you think of the relation of thyroid function to the occurrence of cystic ovaries following your progestin treatment in terms of the results of Leathem's experiments?

DR. HANSEL: On the first one, is there a seasonal difference? I really have no information.

Does someone have some real data on the incidence of cysts in untreated animals?

DR. NALBANDOV: There are some English data.

DR. HANSEL: Can you tell us the results?

DR. NALBANDOV: No, except that there is a very distinct difference between, roughly speaking, the winter months and the summer and spring months. A highly significant difference.

DR. HANSEL: Doesn't Lamond have some data showing some differences in progesterone content of corpora lutea in different seasons of the year?

DR. DONALDSON: The gentleman happens to be here. Perhaps he will answer your question.

DR. LAMOND: Thank you, I thought I would probably get out of this one, but I didn't.

I have done two major experiments in sheep and cattle, in which we have examined the effects of progesterone on the interval from the final injection to the onset of estrus. And we find significant seasonal differences in both cattle and sheep.

This will be given in more detail, I think, in an article in the Journal of Reproduction and Fertility.

DR. HANSEL: In which direction were the differences in terms of the seasons in Australia?

DR. LAMOND. Progesterone has a greater suppressive action in the sheep during the period from June through to August, which is early winter, I take it, and that is about what you would expect. In the cow, it is the same actual months of the year, or in our March to June which is near spring. Let me think about this.

The way I presented the data was in a graph in which I plotted a kind of a score, a reproduction score. It actually indicates that the greatest suppressive action of progesterone in the cow is in our autumn and early winter, and in the sheep is in our early winter and midwinter period. This is quite a clearcut effect of progesterone, both on the actual suppression of ovarian cycles and on the interval to onset of estrus, and also, I suspect, on cystic ovaries.

DR. HANSEL: Very good. Now, there was more to that question, Bob?

DR. MELAMPY: Well, I wonder whether you think we are dealing with any thyroid problems, such as in Leathem's experiments on the rat where he has been able to modify the cystic ovary situation in terms of thyroid function. Do you think that could be involved here in the seasonal problem dealing with pigs?

DR. HANSEL: It could be. There are some data coming from Dr. Louw in South Africa in which he says that there is a reciprocal relationship between TSH secretion and gonadotrophin secretion.

When he feeds lambs stilbestrol he inhibits gonadotrophin content of the pituitaries and increases the TSH content and the thyroid activity in the ewes, as measured by increased epithelial cell height, greater oxygen consumption, and higher blood glucose levels. More recently he has claimed that the reverse is true. There may well be an interrelationship between TSH and gonadotrophin secretion.

Would someone else in the audience like to comment to that point? Dr. Louw's data will be presented at the conference at Trento, Italy, in September, I understand.

DR. SPIES: I would like to ask one of the three speakers, if I am not infringing upon Dr. Casida's and Dr. Dziuk's information to be presented this afternoon, as to whether one of you would care to speculate on the reason for the rebound effect when you compare or combine first and second services in both the sheep and the cow. Is this low effect at first service due to an effect on the ovary, systemic hormone imbalance, or what?

DR. HANSEL: Bob, you haven't said much for awhile.

DR. ZIMBELMAN: Your question, basically, is why is the conception rate reduced sometimes at the synchronized estrus? I don't know.

DR. SPIES: Why do you get the rebounding effect at second service?

DR. ZIMBELMAN: And why is there a tendency for perhaps even an increase in conception rate at second service? I don't have any real data. I sort of subscribe, I guess, to the theory that perhaps there might be a lingering of some progestational effects in the uterus, but I don't know on what basis I tend to view this. Whether it is an effect on the ovary, thinking perhaps of altered follicle development or ovum maturation. I don't really know, Harold. I have really no basis for speculating.

DR. MELAMPY: I would like to ask, what do you mean by lingering progestational activity in the uterus?

DR. ZIMBELMAN: I'll withdraw that remark. Undoubtedly these compounds do have progestational effects.

One point I wanted to make in my own presentation is that the treated animal, as far as I can see, is not only under a high level of progestational influence but is also, in a sense, hyperestrogenic. I can conceive of the uterus as receiving a stimulation with both progestational and estrogenic response and usually, when one tests the response of the uterus to a progestogen, he finds it has increased with the high levels of estrogens or with added estrogens. Perhaps the pituitary or hypothalamus, or the mechanism which then allows the animal to ovulate, recovers more quickly than the uterus from this stimulation.

Perhaps some people have studied these uteri histologically and would care to comment. I couldn't comment even if I saw the slides because I don't know much about this area, but this was the thing I was considering. And I would repeat that this is just a concept on which no data has been developed.

DR. HANSEL: I will go a little way out on a limb there, Bob. Recently, we have been studying the histology of uteri from heifers--control, MAP-treated heifers, and CAP-treated--at 3 days and 10 days after breeding. In other words, at about 6 days after the last feeding and about 13 days after the last feeding.

It does appear that there is some endometrial hyperplasia at three days after breeding. The uterus appears a little more hyperplastic in the treated animals which is, I suppose, a lingering progestational effect. Numbers are still small on this experiment and I don't want to go too far out on a limb.

DR. MARION: Bill, are you implying that this influence is not present at 10 days?

DR. HANSEL: Yes, insofar as the limited data allow.

DR. MARION: I would say, from the histological information we have on the influences of these compounds on the uterine epithelium as well as the glandular epithelium, that this influence is definitely not a progestational one alone. I would be inclined to agree somewhat with the comments Bob made, to the effect that there is a very definite interaction between progestational influences and probable estrogenic functions. The cells typically show estrogen stimulation when compared with both normal tissues from cows during the estrous period and also with tissue from ovariectomized cows under replacement therapy regimes of either estradiol or ECP. The tissue is a little more representative of an estrogen stimulation. Whether or not this estrogen is produced by what we would consider to be normal follicles is a bit difficult to say.

I might make a further comment concerning the statement that Harold brought up. I think he has recently seen some slides presented by one of our students which would give part of the answer to his question. There is a very definite influence of these compounds on the numbers of normal-appearing follicles in the ovary, regardless of the treatment. I suspect we have a few thousand slides to verify this. The number of follicles that are of ovulatory size that would probably ovulate and be considered normal follicles is very definitely reduced during and shortly after progestational influences. The extent of histological changes vary with the compound used. Here we are referring to the differences we find with MGA, for example, where a greater degree of luteinization occurs as compared to the MAP compound. However, the number of follicles that would be considered normal, either with normal granulosa cells or with a normal appearing nonstimulated theca interna layer, is very definitely influenced by these compounds. Perhaps what happens is that, even though ovulation does occur, the conception rate is considerably lower because of the abnormal number of follicles. By the second synchronized estrus this has been eliminated, or at least part of the influence has been eliminated. However, if the influence is not only on ovulatory-sized follicles but also on secondary follicles, follicles that have yet to even produce antra, a considerable reduction in number may occur.

DR. HANSEL: Very good, Gerry. Did you use fairly large doses?

DR. MARION: Yes.

DR. HANSEL: What were your dose sizes?

DR. MARION: Originally we used 200 mg. of MAP in most of our work. However, the MGA[1] studies have varied from 0.4 mg. a day to 1 mg.; 0.4, 0.6, and 1 mg. per cow per day treatments.

DR. HANSEL: Thank you. Bob, do you want to make a comment?

DR. ZIMBELMAN: One point concerns me a little when we start making comparisons of compounds or effects like this and this is one that I tried to make in my paper. We have variations in the conception rate as I indicated earlier--of 25 percent in one particular trial to 75 percent in another. I think it is obvious that when 75 percent of the animals conceive, there couldn't have been many differences from the usual situation. In the case where 25 percent conceived then there are some possibilities, whether it is in the uterus or the luteotrophic circuit. I think that we must be very careful when we are talking about using different compounds to limit ourselves to situations where we have compared them simultaneously; otherwise the comparisons are completely invalid. Undoubtedly the conclusions he might draw after a MAP trial in which he has gotten a 75 percent rate would be different than when he used MAP and got a 25 percent conception as we did in some instances.

I would like to ask if this sort of control was present in your studies and, if not, if you would perhaps care to qualify the results to this extent?

I think the thing we should really attempt to do in the future is to be a lot more specific on the kind of control we have here before we make some of these comparisons. They can be rather upsetting if they aren't made correctly.

I do feel as if we are perhaps infringing on the next section of this program. I hope we aren't taking in too much of Dr. Casida's territory.

DR. HANSEL: Dr. Casida has a comment at the moment.

DR. CASIDA: I am interested in Dr. Zimbelman's data on the evidence of estrogen production while MAP was being fed. I am lost though as to the implication of it. Is this simply a function of dose so there is not complete suppression of follicular development, or do you mean to imply that there may be some stimulation of follicular development by MAP. Just what is behind this?

DR. HANSEL: A very good question.

DR. ZIMBELMAN: I'm sure the answer won't be nearly as good.

The question also relates to one of Dr. Donaldson's that I have here and have been pondering. If you recall the first slides, we showed the corpus luteum regression and then later illustrated increased follicular development. Ths was essentially my concept of the development that occurs. I don't anticipate saying that MAP stimulates gonadotrophin release but rather that in preventing these follicles from ovulating it allows them to develop further. I had intended to comment on this and I think my impression is that there is perhaps very little influence on FSH release by these compounds, but they allow greater follicular development to occur. In terms of LH, I favor a concept that perhaps I am somewhat enchanted with, in which we have two centers for LH release, one for chronic LH release which perhaps is not being inhibited by the MAP and another for cyclic LH release causing ovulation. Therefore, we get the LH in low levels and FSH interacting to cause larger follicles and perhaps estrogen production. The oral progestogens perhaps block the cyclic release of LH which normally would cause ovulation. In terms of estrogenicity, I think we have only the gross observations, such as increased activity of the animals, increased mucus, uterine tone, vulvar swelling, and things of that nature when the animals are being inhibited. Estrogenicity is confirmed by the cervical mucus smear ratings, which do increase. I am assuming that this is due to an increased estrogen activity.

DR. CASIDA: Do you have any evidence of a negative correlation between the amount of this and dose?

DR. ZIMBELMAN: No, I do not have good data on the effect of dose on follicular fluid weight but in terms of the gross observations as determined by palpation, my impression is that even at 10 times the minimal dose which blocks ovulation we still get a good deal of follicular development. I feel quite sure that one could show a dose relationship. I think that the dose required to inhibit the follicles--this is just based on palpation observations--to a stage where they might not be palpable through a large part of the estrual cycle would produce an interval of 10 days or longer from the last feeding until the animal returns to estrus. I think Dr. Wagner, Dr. Hansel, and others have shown as you increase the dose you increase the interval from the last feeding to estrus. I think to get follicular suppression with the progestogen you produce extremely long delays from the last feeding to estrus. It takes this high a dose level. There probably is some dose relationship at the near minimal dosage for inhibiting ovulation. It is in this area that I have been talking about where the estrogenic influence exists, as I conceive of it, at least.

---

[1] MGA is melengestrol acetate. The Upjohn Co., Kalamazoo, Mich.

DR. HULET: Dr. Foote, of Utah, who doesn't appear to be here, and I have been doing some work in cooperation which will be reported at the Western Section Meetings, which I would think is pertinent to your question, Dr. Casida.

We have given two levels of MAP, 8 mg. and 16 mg., to ewes over a 20-day period. As I recall, on the 16th day of this period, we have given 1,200 I.U. of PMS to both of these groups. In addition, each of these groups has been subdivided and given estradiol-17 beta. I have forgotten the dosage. We found that there is little difference in the follicular development at these two different levels of MAP. However, ovulation rate is almost completely suppressed at the 16 mg. level but is only slightly suppressed at the 8 mg. level. And at the 16 mg. level with the estradiol the ovulation suppression is only very slight. It is comparable to the PMS given while under the influence of the 8 mg. MAP level. I hope that is of some help.

DR. CASIDA: How do you interpret that?

DR. HULET: I am not sure that we have the interpretation. I hope Dr. Foote will have the interpretation when he presents the paper.

DR. HANSEL: I would suspect before this meeting is over someone is going to suggest that MAP or CAP, instead of inhibiting LH release, may actually stimulate it in a chronic way. I don't want to be the first to do this.

DR. DUTT: I would like to ask if anyone has any data on long-term feeding of either MAP or CAP? In regard to the question of fertility, we have some data showing that following a single subcutaneous injection in ewes and we can consistently get a cystic (we call it a cystic) uterus. We have drawn off as much as 2 liters of fluid from these uteri. We have similar observations in beef heifers.

DR. HANSEL: What levels were these injections?

DR. DUTT: We used 50 mg. of MAP in ewes in a single injection and a single 200 mg. injection in heifers.

DR. HANSEL: A condition of hydrometria.

DR. DUTT: I wonder if anyone has really taken a good look at this following feeding or has any long-term feeding experiment been done?

DR. HANSEL: Someone has a long-term feeding experiment. Who is it?

DR. WAGNER: Not in ewes, but in cattle. The experiment was designed as a toxicology experiment. We wanted to treat cattle for an extremely long period of time. I am sure we are all familiar with some of the problems resulting from using hormones and the Federal Government's concern with hormone residues. Ten mg. of MAP was fed for 168 days. Our original intention was to watch the animals for 5 or 6 days following removal and then turn bulls in with them and slaughter in 150-200 days and see whether or not we could finally get pregnancies. Only gross observations were made and we are not implying that the heat or the reproductive phenomena following removal were in any way, shape, or form normal. However, we did synchronize 75 percent of these animals. We had 40 animals and I think 10 of these conceived at the first service, and a total of 8 percent conceived following two services, results somewhat similar to Dr. Zimbelman's 26-day data. Is the uterus adversely affected following this long period? And if it is, for how long? Well, I would say not more than few days. Otherwise we would have not had an 86 percent conception 26 days following treatment. We thought we would have a long period of time before recovery, but did not.

DR. HANSEL: It is a matter of level and route of administration?

FOREST JUDGE: I have a question. Where does this synchronized estrus occur after such long-term feeding?

DR. WAGNER: As you know, we have been presenting our data with an average interval and a standard deviation from last feeding. These data show no difference from other treatments. Actually there was a difference, because of the fact that only one of the 40 animals came in heat on the 3d day, and then about 25 came in heat on the 4th day. Thus our average interval and our standard deviation were about the same as we had been seeing, but actually we felt that the long treatment delayed them a day.

DR. HANSEL: But essentially they were not greatly different from the short-term treatment.

DR. WAGNER: Not greatly different.

DR. SORENSON: I would like to make one observation that we did on some MAP feeding for a 32-day period. First, let me give the reasons for doing this. From a practical standpoint some of our ranches would like to bring their cattle in as they find them in estrus and put them on feed, so that this would be some way that they would know when they were started. They wouldn't be just starting anywhere in the cycle. With this in mind we put some on MAP in the feedlot for 32 days. We had some animals that we slaughtered at 2, 4, 8, 16, and 32 days. The gross observations are all I can give you now. The tissue is in the process of being sliced. The uterus went into a progestational state. That is, it became rather flaccid, and lacked tone. The ovaries had numerous small follicles, throughout the 4, 8, 16, on up to the 32-day stage. We found at 32 days that the uterus took on a tone that we could call almost an estrous tone. This was completely different from the 16-day uterus. At 16 days we still had the soft progestational type we would expect but at 32 days we had a firm estrous type, that is, an estrous feeling type of uterus. As soon as we get the tissue out we'll try to tell you about it, but I think the results are interesting.

DR. HANSEL: Very good.

DR. GERRITS: I have a comment to make. We have been talking all morning about steroids and their effect of blocking of the estrous cycle and controlling of the estrous cycle.

At Beltsville we have been doing a little work with a non-steroidal compound. I thought I might at least mention briefly some of the results that we have obtained.

The compound we have been using is a hydrazine derivative. In the initial trial 34 animals were subjected to treatment. Twenty-two of 26 gilts fed approximately .8 to 1.5 mg. per kg. of body weight per day expressed estrus on the 5th, 6th, or 7th day after withdrawal. The conception rate was 73% based on cleaved eggs recovered at 3 days post breeding or embryos recovered at more advanced stages of gestation. A paper that will include the details of this study will be presented at the Fifth International Congress on Animal Reproduction and Artificial Insemination in Trento, Italy. I did't mean to interrupt the discussion on the orally active progestogens. However, since we are near the end of the section of the conference that deals with evaluation of methods to control the estrous cycle. I thought a mention of this work would be appropriate at this time.

DR. HANSEL: Well, thank you very much. This is an extremely interesting observation. I included, as did several others, in my papers a plea for studies with compounds of different types.

DR. SORENSON: Ray Dutt was asking about fluid awhile ago and this is one of the reasons I wanted to say what I did and then I left it out. We did not see any more fluid in these animals through these stages of 4, 8, 16, 32 days. We did not see any change in fluid content of the uterus.

SOME SIDE-EFFECTS AND AFTER-EFFECTS OF EXPERIMENTAL ENDOCRINE TREATMENTS USED TO MODIFY THE NATURAL ESTRUAL RHYTHM[1]

L. E. Casida
University of Wisconsin

The side-effects and after-effects to be considered in this discussion are those which grow out of experimental attempts to modify the natural estrual rhythm, and which have a bearing on the subsequent fertility of the animal. They are the effects that at one-and-the-same-time are the despair and the hope of workers on technological control of reproduction. They are the despair in that they detract from our immediate ability to control reproduction; they are the hope in that we may be able to learn more about the reproductive process in our attempts to analyze and understand them.

At least four ways have been suggested for altering or controlling the estrual cycle and the consequent breeding time in livestock.

1) The induction of ovulation and the initiation of pregnancy as the direct result of treatment with gonadotropic hormones.
2) Induction of new corpora lutea in all animals of the herd at the same time by injection of gonadotropins. The estrual cycle in the different animals then taking as its time of departure the formation of the new corpora lutea, and all animals coming back into estrus at the same time.
3) Inhibition of ovulation in the different animals of the herd, simultaneously, for such a minimal period of time as is necessary for the ovaries of all animals to become free of functional luteal tissue. Cessation of the inhibitory treatment then being followed by estrus in all animals at approximately the same time.
4) Destruction of the corpora lutea in the different animals at the same time, no matter what the stage of their development with the consequent return of all animals into estrus at the same time.

The first of these presumes that ovulation and fertility can be brought about regardless of the stage of the estrual cycle at the time of treatment.

The second of these assumes that the new corpora lutea take command of the cycle and that the old corpora lutea either regress at their initially appointed time or that they continue on and then regress at the time of the new corpora lutea presumably one estrual cycle length from the time of the induction of ovulation. It, of course, is hoped that the different animals will respond very much alike to this treatment so that the date at which they come back into estrus after regression of the newly formed corpora lutea will show much less variation than the initially anticipated time of estrus.

The third suggested method implies the application of the treatment at different stages of the cycle and inhibition of estrus for different lengths of time in the different animals. In general, those animals in which treatment is initiated early in the cycle will be held out of the anticipated estrus the shortest time. Those later in the cycle will be held out for a longer time; there is a stage late enough, however, that there is no interference with the next estrus and treatment will have ceased before the following one. It is presumed again that the interruptions and modifications of the normal physiology will leave no imbalance resulting in aberrations leading to lowered fertility.

In the fourth instance, instead of the cycle being lengthened as in the third, it is being shortened by destruction of the corpora lutea. It is the hope that the method of destroying the corpora lutea will not be generally harmful to the health of the individual nor lessen the fertility of the return estrus.

For all of the four suggested methods of altering the estrual cycle and controlling the breeding time there are good theoretical reasons expecting them to work. The danger, however, is that unanticipated side-effects or after-effects, not necessarily injurious to the animals' health, may affect the immediate or the subsequent fertility of the animal.

It was early shown in cattle (8) that ovulation can be induced at most any stage of the estrual cycle, and during the post-partum interval. It was found, however, that in many of these individuals insemination did not lead to pregnancy. In fact it did not lead to fertilization of the eggs except as ovulation was brought about during the follicular phase of the cycle very near to the time of expected estrus. Similar results were demonstrated in the sheep (18) and also in the rabbit (19). In general, eggs that were ovulated during the follicular phase of the cycle were fertilizable and those during the luteal phase were not fertilizable.

Further studies with the rabbit (17) indicated that eggs ovulated during the luteal phase of the cycle could be fertilized if the semen were introduced directly into the uterus rather than being deposited into the vagina. Similar attempts in the cow (6) and in the pig (26) also showed fertility for the luteal phase eggs but in general the fertilization rate was low and in al three species the uterine insemination in the luteal phase of the cycle quite commonly led to the development of pyometra (6). The condition was explored further in the rabbit (3) and it was found that although fertilization could be accomplished, embryo survival was particularly low, presumably because of the pyometra that had been induced by insemination. This was believed so because if the young embryos were removed from the oviducts of the luteal phase animals that had not been inseminated, the embryos survived.

---

[1] Paper No. 972 from the Division of Genetics.

It was shown also in the rabbit (4) that administration of progesterone to the follicular-phase animal effected a marked depression in the fertilization rate. The explanation of this depressing effect of progesterone may lie in part in the poor transport of sperm and possibly in poor capacitation of the sperm.

When new corpora lutea are induced in all animals of the herd at the same time, it is hoped that the interval to next estrus may be more uniformly related to the time of the formation of the new corpora lutea than to the time of the preceding estrus. Attempts to regularize the interval to next estrus in sheep by the injection of gonadotropic hormones at different stages of the estrual cycle (7) have given different results depending upon the time at which the sheep were injected and depending upon the kind of gonadotropic preparation. Initial studies were made by subcutaneous injection with a purified follicle stimulating extract to increase the number of ovulable follicles, and following this by intravenous injection of an unfractionated mixture of FSH and LH. Other sheep were injected subcutaneously with unfractionated extracts to grow a larger number of follicles and followed then by an intravenous injection of the same preparation to produce ovulation. Subcutaneous treatment with unfractionated extracts increased the intervals to the next estrus to an average of 25 to 31 days depending on whether the treatment terminated on the 9th or the 13th days of the estrual cycle. The variation in length of cycle was increased tremendously so that in no sense was there a regularization of the interval nor was there a timing of next estrus to a normal cycle length from the time of induced ovulation. When the number of follicles that responded had been increased by giving FSH rather than unfractionated extract, the results differed depending on the stage at which the treatment was given. The time of the next estrus and the variability in the time that the next estrus occurred were virtually unaffected when treatment terminated on the 9th day of the estrual cycle, but estrus was delayed on the average and the variability was increased greatly when treatment terminated on the 13th day.

The delay in the next estrus and the variability in the time of next estrus were probably traceable to the formation of a number of cystic follicles and luteinized cysts and these were more prevalent with the unfractionated extract than with the follicle stimulating preparation. With both preparations, however, there were instances of anestrual intervals of more than 60-70 days with one ovary still containing more than 10 functional-appearing corpora lutea and the other ovary only regressed corpora lutea. There may have been recurrent superovulation after treatment in these cases or there may have been persistence of corpora lutea on a local basis for an unusual length of time.

There is also the work with sheep (13) where ovulation was induced by injection of fairly minimal amounts of HCG or of unfractionated extracts of the pituitary gland. In these cases, the induction of new corpora lutea at different stages of the cycle with a minimum of follicle stimulation has had no effect on the interval to the next estrus. The mere induction of ovulation does not appear to disturb the rhythm of the estrual cycle but if superovulation is induced as in the earlier studies, there may be a sequence of events which leads to the formation of accessory corpora lutea, follicular cysts, and luteinized follicular cysts all of which tend to delay the next estrus for a variable length of time. This has been shown particularly in trials with swine (21) in which ovulation was induced at different stages of the cycle with HCG. The new ovulations were marked for identification while the corpora were young, but when the animals were slaughtered at the 25th day there were several instances in which additional corpora lutea had been formed after the time that the new corpora had been marked. Sometimes there had been regression of the old corpora and sometimes not. A reaction was set up which led to the formation of additional corpora lutea with the original maintenance mechanisms being disrupted markedly.

The initial study on the inhibition of ovulation in farm animals by administration of progesterone (9) demonstrated many aspects of our present knowledge of the effects of this treatment. Sub-optimal dosage showed a tendency to permit ovulation during the course of treatment. There was also a greater incidence of cystic follicles at the end of treatment than with a more optimal dosage. The time of recurrence of estrus was earlier and in general the fertility level was lower when the sub-optimal dosage was used. Later and more complete analysis of dosage effects (10) has shown that the lowest levels fail to inhibit estrus and ovulation. At a higher level there is inhibition of estrus and ovulation with the formation of cystic follicles during treatment. At a still higher level, there is inhibition of follicular development, estrus, and ovulation during treatment with a uniform post-treatment estrus and ovulation. At the highest levels there is an extreme inhibition of follicular development during treatment, with a marked delay or an absence of estrus following treatment. During the delay interval there is a strong tendency for cystic follicles to develop.

Species differences appear to exist in the efficacy with which progesterone may be used to control the occurrence of estrus in a group of animals. The initial study on synchronization of lambing and shortening of the lambing interval (20) gave results that were quite clear-cut and there was no lessening of fertility. Other studies (27) with sheep and to a certain extent with cattle, particularly when progesterone was injected, have borne out much of the original promise. In general, the degree of synchronization in swine and the fertility level have been lower than with the ruminants, particularly sheep.

Evidence as to how progesterone administration may affect fertility in swine (1) is indicated by an increased percentage of eggs lost as measured by the discrepancy between number of corpora lutea and number of recovered ova. Also, there was a greater percentage of unfertilized eggs, but embryonic death did not appear to be increased.

That high dosages of progesterone may have a latent affect on the pituitary-ovarian relationship (14) was demonstrated in the lack of change in pituitary gonadotropin content during the post-treatment period despite the fact that follicular development was increasing steadily with cyst production. This is in contrast to the decline in pituitary gonadotropin (11) concomitant with the resumption of normal ovarian activity in the period following a modest course of treatment with progesterone injections.

A clinical practice in treating cows showing absence of estrus is the manual removal of the corpus luteum from the ovaries by way of the rectum. Estrus often occurs within a few days after this removal. This is about the only technique available at the present time for effecting the destruction of corpora lutea in different animals at the same time. Removal of the corpora lutea in the ewe (13) at days 5, 9, or 13 leads to return of estrus in 2 to 3½ days. Estrus recurs after 2 to 6 days in most normal cows when corpora are removed at days 7-17 of the cycle (Unpublished data, University of Wisconsin). A very small amount of data (23) exists for the pig and it suggests a slightly longer interval before estrus resumes. Another possible approach to destruction of the corpus luteum (28, 24, 16) is to interfere with its formation so that it may be maintained for a shorter than normal time. There is evidence that administration of progesterone to the sheep, pig, and cow in the early part of the cycle results in smaller and less well developed glands. There seems to be little data, however, whether such corpora have shorter than normal life.

The likelihood of the animal developing cystic ovaries following the heat after removal of the corpus luteum appears to be somewhat enhanced (Unpublished data, University of Wisconsin). This likelihood may be increased even further if two or more corpora lutea are removed in succession (12). A similar phenomenon was shown also (5) in swine from which part of the corpora lutea were removed by unilateral ovariectomy; the incidence of cystic ovaries was increased.

Another experimental modification of the normal reproductive cycle which probably increases the chances of cyst formation is the removal of pigs at birth. Baker *et al.* (2) showed a rather unusual incidence of cystic ovaries in animals so treated as did also Self and Grummer (22) when litters were weaned at 10 days of age. More recent data (21) have shown a higher incidence of cystic ovaries in sows from which the pigs were removed at birth than in those from which pigs were removed at 21 days.

Various treatments in which suppression of ovarian activity has been removed suddenly, whether it be by withdrawal of heavy doses of progesterone or by removal of corpora lutea or by parturition without the inhibitory followup of nursing, have resulted in increased probability of ovarian disfunction.

Finally, consideration will be given to certain experimental situations which seem characterized by low fertilization rates. It was shown in sheep (18) that ovulation could be induced during anestrus by injecting gonadotropic hormones. Estrus did not occur in these animals but the fertilization occurrence was virtually nil when artificial insemination was performed near the time of ovulation.

Another illustration has been shown in beef cattle (15) where ovulation has been induced rather regularly by injections of gonadotropic hormone approximately two weeks after calving. The fertility of these eggs following artificial insemination also appears to be nil. A similar low fertilization rate of eggs from sows treated with gonadotropins shortly after parturition (21) has also been demonstrated. Making eggs available for fertilization without the occurrence of estrus has not led to appreciable fertility, with the exception noted earlier at the time of the luteal phase of the cycle when fertilization might be produced by insemination into the uterus. In that instance, however, the best results have been obtained in the rabbit where the intravenous injection of the gonadotropin causes regression of the corpus luteum with a presumed quick decline in progesterone (25).

I would like to include an afterthought with the last point in the prepared paper. The tests that have been made for increased embryo mortality must be considered insensitive relative to the importance of such possible after-effects. Little concern appears to have been given members of the next generation, other than to get them here and as many of them at the same time as possible by synchronization.

We might ask what weaknesses or strengths in the reproduction process may evolve genetically as natural selection between females plays its role in an environment where hormonal synchronizers act as ecological factors; also with the approach to control of estrus which lengthens the interval of time that the ovum spends in the vesicular follicle, what defects may arise in the germ plasma through aging? or through prolongation of a period of increased sensitivity to mutagenic factors?

I would like to emphasize these are areas that we need to be watching to see if there is any evidence of effects from the standpoint of selection of females that will respond to synchronization and whether prolonged delay of estrus does affect the viability and quality of the offspring produced.

Now we will continue the discussion. Dr. Dziuk will present the material from the University of Illinois which they have been gathering now for a number of years and then, following that, we will open the discussion.

Summary

Side-effects and after-effects of treatment which affect fertility have occurred with all of the experimental approaches to control of the estrual cycle. Fertilization failure and formation of cystic ovaries are the most common and there is relatively less evidence for increased embryo mortality.

Literature Cited

(1) Baker, L. N., Ulberg, L. C., Grummer, R. H., and Casida, L. E.
1954. Inhibition of Heat by Progesterone and Its Effect on Subsequent Fertility in Gilts. Jour. Anim. Sci. 13: 648.

(2) Baker, L. N., Woehling, H. L., Casida, L. E., and Grummer, R. H.
1953. Occurrence of Estrus in Sows Following Parturition. Jour. Anim. Sci. 12: 33.

(3) Black, W. G., Otto, G., and Casida, L. E.
1951. Embryonic Mortality in Pregnancies Induced in Rabbits of Different Reproductive Stages. Endocrinology 49: 237.

(4) Boyarsky, Lila H., Baylies, Harriet, Casida, L. E., and Meyer, R. K.
1947. Influence of Progesterone Upon the Fertility of Gonadotropin-Treated Female Rabbits. Endocrinology 41: 312.

(5) Brinkley, H. J., Wickersham, E. W., First, N. L., and Casida, L. E.
1964. Effect of Unilateral Ovariectomy on the Structure and Function of the Corpora Lutea of the Pig. Endocrinology 74: 462.

(6) Casida, L. E.
1953. Some Factors Affecting Fertilization and Embryonic Death. Ciba Foundation Symposium Mammalian Germ Cells. G. E. W. Wolstenholme, Ed., pp. 262-274.

(7) Casida, L. E., Dutt, R. H., and Meyer, R. K.
1945. Alteration of the Estrual Cycle by Pituitary Gonadotropins and Persistence of the Effects upon Reproductive Performance in Ewes. Jour. Anim. Sci. 4: 24.

(8) Casida, L. E., Meyer, R. K., McShan, W. H., and Wisnicky, Walter
1943. Effects of Pituitary Gonadotropins on the Ovaries and the Induction of Superfecundity in Cattle. Amer. Jour. Vet. Res. 4: 76.

(9) Dutt, R. H. and Casida, L. E.
1948. Alteration of the Estrual Cycle in Sheep by use of Progesterone and its Effects upon Subsequent Ovulation and Fertility. Endocrinology 43: 208.

(10) First, N. L., Stratman, F. W., Rigor, E. M., and Casida, L. E.
1963. Factors Affecting Ovulation and Follicular Cyst Formation in Sows and Gilts Fed 6-Methyl-17-Acetoxyprogesterone. Jour. Anim. Sci. 22: 66.

(11) Foote, W. C., Waldorf, D. P., Self, H. L., and Casida, L. E.
1958. Some Effects of Progesterone and Estradiol on the Ovarian Structures and on the Gonadotropic Potency of the Pituitary Gland of the Gilt. Jour. Anim. Sci. 17: 534

(12) Foote, W. D., Zimbelman, R. G., Loy, R. G., and Casida, L. E.
1959. Endocrine Activity of Corpora Lutea from First Service and Repeat-Breeder Dairy Heifers. Jour. Dairy Sci. 42: 1944.

(13) Inskeep, E. K., Oloufa, M. M., Pope, A. L., and Casida, L. E.
1963. Functional Capabilities of Experimentally Induced Corpora Lutea in Ewes. Jour. Anim. Sci. 22: 159.

(14) Kirkpatrick, R. L., First, N. L., and Casida, L. E.
1963. Effect of Estradiol-17 on Follicular Development and Pituitary

Potency in Gilts Fed 6-Methyl-17-Acetoxyprogesterone. Jour. Anim. Sci. 22: 767.

(15) Lauderdale, J. W., Jr.
1964. Unpublished Data. University of Wisconsin.

(16) Loy, R. G., Zimbelman, R. G., and Casida, L. E.
1960. Effects of Injected Ovarian Hormones on the Corpus Luteum of the Estrual Cycle in Cattle. Jour. Anim. Sci. 19: 175.

(17) Murphree, R. L., Black, W. G., Otto, G., and Casida, L. E.
1951. Effect of Site of Insemination Upon the Fertility of Gonadotropin-Treated Rabbits of Different Reproductive Stages. Endocrinology 49: 474.

(18) Murphree, R. L., Warwick, E. J., Casida, L. E., and McShan, W. H.
1944. Potential Fertility of Ova from Ewes Treated with Gonadotropins. Jour. Anim. Sci. 3: 12.

(19) Murphree, R. L., Warwick, E. J., Casida, L. E. and McShan, W. H.
1947. Influence of Reproductive Stage Upon the Fertility of Gonadotropin-Treated Female Rabbits. Endocrinology. 41: 308.

(20) O'Mary, C. C., Pope, A. L., and Casida, L. E.
1950. The Use of Progesterone in the Synchronization of the Estrual Period in a Group of Ewes and the Effect on their Subsequent Lambing Record. Jour. Anim. Sci. 9: 499.

(21) Peters, J. B. and Short, R. E.
1964. Unpublished Data. University of Wisconsin.

(22) Self, H. L. and Grummer, R. H.
1958. The Rate and Economy of Pig Gains in the Reproductive Behavior in Sows When Litters are Weaned at 10 Days, 21 Days or 56 Days of Age. Jour. Anim. Sci. 17: 862.

(23) Short, R. E. and Peters, J. B.
1964. Unpublished Data. University of Wisconsin.

(24) Spies, H. G., Zimmerman, D. R., Self, H. L., and Casida, L. E.
1959. The Effect of Exogenous Progesterone on Formation and Maintenance of the Corpora Lutea and on Early Embryo Survival in Pregnant Swine. Jour. Anim. Sci. 18: 163.

(25) Stormshak, F. and Casida, L. E.
1964. Effect of Gonadotropins on Corpora Lutea of Pseudopregnant Rabbits. Endocrinology. (In press.)

(26) Tanabe, T. Y., Warnick, A. C., Casida, L. E., and Grummer, R. H.
1949. The Effect of Gonadotropins Administered to Sows and Gilts During Different Stages of the Estrual Cycle. Jour. Anim. Sci. 8: 550.

(27) Ulberg, L. C.
1955. Synchronization of Estrus Cycles. Proc. Cent. II Symposium Reproduction and Infertility, page 104. Mich. State Univ.

(28) Zimbelman, R. G., Pope, A. L., and Casida, L. E.
1959. Effect of Progesterone on the Corpus Luteum of the Bred Ewe. Jour. Anim. Sci. 18: 1327.

RESPONSE OF SHEEP AND SWINE TO TREATMENTS FOR CONTROL OF OVULATION[1]

P. J. Dziuk
Illinois Agricultural Experiment Station, Urbana

Control of the estrous cycle is essential to precise research in gamete and zygote physiology and could be useful in animal husbandry.

The conception rate in sheep during the breeding season following treatments to control ovulation has varied from about normal (Dauzier, 4; Robinson, 20; Evans et al., 9; Lamond and Bindon, 17; Brunner et al., 3; Hinds et al., 15), to distressingly low levels in other cases (Braden et al., 2; Davies, 5; Lamond, 16). Treatments during the anestrous season have given a variable response but usually only 30 to 50 percent of ewes lamb (Gordon, 13; 14).

In swine, treatment by either injected progesterone or orally administered progestogens has usually caused cystic follicles, only partial control over ovulation, and low fertility (Baker et al., 1; Nellor, 18; Nellor et al., 19; First et al., 10); Gerrits et al., (12) found no adverse effects on fertility from injected progesterone. High levels of progestogens followed by an ovulating gonadotrophin controlled ovulation time but incidence of heat and fertility was low (Dziuk and Baker, 6).

The following report is a compilation of published and unpublished research on sheep and swine to control the estrous cycle.

Materials and Methods

Sheep: Seven hundred aged ewes with a predominance of Merino breeding were treated in groups of 5 or 10. They were killed by electrocution at a prescribed time and examined for follicular growth, ovulation points, and fertile eggs or embryos. An additional 600 ewes were treated in groups of 10 to 40 and allowed to lamb. These ewes were of a variety of backgrounds. Most were mated to a specific ram and his fertility was determined. Six-methyl-17-acetoxyprogesterone (MAP) was administered for 14 days as part of the daily diet, usually at 50 to 75 mg. daily per ewe. Injections of pregnant mare's serum gonadotrophin (PMS) were made subcutaneously (SQ) just behind a front leg at levels of 500 I.U. per ewe. Human chorionic gonadotrophin (HCG) was given intramuscularly (IM) at either 250 I.U. or 500 I.U. per ewe. Estradiol cyclopentylproprionate (ECP) was given intravenously (IV) as an oily solution at 1 or 2 mg. per ewe.

Swine: Two hundred fifty-eight gilts and sows in 14 different groups were fed 120 mg. MAP per day per female for 18 days. Each group also had untreated controls of the same background mated to the same boars as a treated group. These groups were at several different farms, under different management systems, at different seasons, and were composed of several different breeds and ages of females. Gilts not pregnant were killed and the appearance of ovaries and uteri noted. Forty-eight prepuberal gilts about six months of age were treated with 500 mg. of MAP for 9 days and observed for heat.

One hundred eighty prepuberal gilts, 5 to 5½ months old, were treated to induce follicle growth and ovulation with mixtures of 125 I.U. of PMS plus 125 I.U. of HCG or 250 I.U. of PMS plus 250 I.U. of HCG, as shown in table 5, or by orally administered ethynil estradiol (EE). They were all checked daily for heat. About half were killed and examined 6 to 8 days after treatment. EE was given orally to each of 10 cycling gilts for 5 days at a level of 16 mg. daily. Seventeen other cycling gilts received 20 mg. EE daily for 15 days. Prior heat dates were known and subsequent ones noted.

Sixty-eight gilts with at least one previous heat were injected with 250 or 500 I.U. of HCG about 24 hours before expected estrus as judged by previous heat date, behavior, and appearance of the vulva. These gilts were killed or laparotomized 2-14 hours after ovulation and examined for ovulation points and the eggs were individually examined as fresh, whole mounts, and in most cases also as fixed and stained preparations.

Results and Discussion

Sheep: Of 288 ewes treated during the breeding season, 275 (95%) showed heat over the 24 hour period beginning 48 hours after last MAP and 162 (59%) lambed to this service. The conception rates among treatment groups were not significantly different. The conception rates among rams ranged from zero to 85% and within treatment groups there were differences due to rams in nearly every case. This points out the importance of recognizing differences among rams when trying to evaluate fertility after a treatment.

While the time of heat was well correlated with the last feeding of progestogen, the

---

[1] The donations of various hormone preparations and other support by the following companies are gratefully acknowledged: The Upjohn Co., Eli Lilly Co., Ayerst Co., and Mead Johnson Co.

correlation between the times of last progestogen feeding and ovulation was very poor (table 1) (Dziuk et al., 8). While 81% (77 of 95) of ewes killed 60-94 hours after last MAP showed heat during this time, only 48% (28 of 58) of ewes killed 80-94 hours after last MAP had ovulated.

A lower proportion (127 of 190, 67%) of anestrous ewes showed heat after treatment, either with or without PMS, than ewes treated during the breeding season. The conception rate to first service of anestrous ewes (52 of 190, 27%) was also lower than the conception rate of ewes treated during the breeding season (269 of 500, 54%). To what extent these differences were due to ram fertility and what extent were due to the ewes is unknown.

The daily administration of 200 mg. of MAP to 20 ewes inhibited heat and ovulation during administration and for at least 5 days after the last feeding. About half the ewes did not show heat for at least 20 days. An examination of the ovaries of representative, treated ewes revealed several different conditions. Some had no follicular development and no corpora lutea, some had follicles 5 to 8 mm. in diameter and no corpora lutea, while others had ovulated but had not shown heat. It is possible that the progestogens were no longer present in the system but that the high levels had in some way affected the hypothalamic-pituitary axis and caused a delayed recovery.

The administration of PMS near the end of MAP treatment did not increase significantly the number of lambs born per pregnant ewe over non-PMS treated ewes. In those experiments in which direct comparisons are possible, there were no statistically significant differences due to time of PMS administration relative to the last MAP feeding (table 2).

Incorporation of HCG with PMS as a follicle stimulator resulted in 78% of ewes showing heat as opposed to 95% of similar ewes not given HCG. Only 37% of ewes receiving HCG with PMS lambed as opposed to 59% of similar ewes not given HCG. Other ewes treated in a similar manner but examined for ovulations had ovulated prematurely as a result of the injection intended to be a follicle stimulating injection. In one experiment neither HCG nor ECP caused high fertility, even though HCG is known to cause ovulation in all ewes similarly treated and ECP causes heat in all ewes (table 3). It may be possible to induce both heat and ovulation by combining the HCG-ECP treatments and thereby increasing fertility.

Swine: Daily administration of 120 mg. of MAP per gilt for 18 days did inhibit heat and ovulation and after withdrawal, heat was synchronized in a certain portion of gilts but conception rate and litter size were significantly smaller than for untreated controls (table 4). Two hundred other gilts not shown in the table were examined after treatment and mating, if it occurred. Sixty, of 200 gilts, had one or more unruptured follicles 15 mm. or larger. Twenty-two of these 60 had only large follicles, 23 had large follicles plus corpora lutea, and 15 of these were pregnant and also had large follicles and corpora lutea of normal appearance. During the course of these experiments, some gilts showed heat and either ovulated very few follicles or failed to ovulate, while some ovulated and did not show heat. There was a great deal of variability in response not readily explained on the basis of obvious differences within and between groups.

Administration of 500 mg. of MAP per gilt daily for 9 days inhibited heat and ovulation. Injection of 500 I.U. of HCG 5 or 6 days after last MAP feeding, caused ovulation in 94% of gilts but heat occurred in only 4% of animals after withdrawal (Dziuk and Baker, 6). Fertility was low, due at least in part to poor sperm transport to the site of fertilization. Fifty or 100 mg. of diethylstilbestrol (DES) injected about 24 hours before insemination did raise the level of fertility of eggs but interfered with implantation (Dziuk and Polge, 7). Forty-eight gilts were treated with 500 mg. MAP daily for 9 days at about 6 months of age but before their first heat. Only 4 showed heat during the following 21 days and very few had shown heat even 60 days later. This indicates a persisting effect on the onset of puberty due to even a short treatment with high levels of MAP.

The oral administration of 16 mg. of EE daily for 5 days to 10 cycling gilts inhibited heat for an additional 12 days presumably by maintaining the existing corpora lutea as shown by Gardner et al., (11). Seventeen cycling gilts were given 20 mg. EE daily for 15 days. None showed heat for the next 40 days. Seven were killed and all had corpora lutea that appeared normal; at 50 days after treatment 5 more were killed and at 80 days the remaining 5 were killed. None showed heat prior to killing and all had corpora lutea. The uteri were enlarged and vascular and had the appearance of a pregnant uterus with the exception that no fetuses were present. If gilts were started on EE treatment during days 15 to 19 of the cycle they came into heat in the next few days as expected, but if treatment started on days 1 to 13 of the cycle they did not show heat.

Twenty prepuberal gilts, 5½ to 6 months of age, were given 20 mg. of EE orally for 5 days. Fifteen were in heat on the 4th, 5th, or 6th day after first treatment. Ten showing heat were examined 8 days after the last EE. Eight had ovulated but had an average number of corpora lutea of only 4 per gilt. Each gilt also had an average of 9 follicles, 4 to 5 mm. in diameter. Only one of the remaining 10 gilts showed heat during the next 60 days, indicating a persisting effect.

A single intramuscular injection of either 250 or 500 I.U. of HCG given to 68 gilts in late proestrus caused ovulation in 67 of these and eggs were recovered after ovulation and used for other studies. The mean number of corpora lutea per gilt was 14.7, indicating that the injection did ovulate the number of follicles that might be expected at a normal ovulation. There was no indication that fertility was affected.

A single subcutaneous injection of a mixture of PMS and HCG given to 180 prepuberal gilts, 5½ to 6 months of age, caused heat 4, 5, or 6 days later in 81 (45%) (table 5). Seventy-two percent of those examined had ovulated. Apparently the single injection of gonadotrophin is a sufficient stimulus to cause follicle growth, heat, and ovulation. Only 8% of animals had a second heat about 21 days later. This treatment then does not initiate regular cycles.

Summary

Sheep: Withdrawal of daily oral administration of 50-75 mg. of MAP to ewes during the breeding season caused synchronous heat in 95% of animals. Fifty-nine percent produced lambs to this heat. The differences among rams in conception rate were greater than differences among usual treatments. Ovulation time was not well correlated with onset of heat in all animals. Treatment of anestrous ewes with MAP alone or with MAP and PMS caused heat in 67% of 190 ewes and 27% of 190 ewes produced lambs to the first service.

High levels (4X) of MAP for 7 days delayed heat and ovulation for at least 5 more days after last MAP treatment. PMS given near the last MAP feeding did not increase the number of lambs born and had no adverse effect. It appears that PMS is equally effective when administered either zero, 24, or 48 hours before the last MAP feeding.

HCG caused ovulation to occur and ECP induced heat but neither increased the conception rate.

Swine: The daily administration of 120 mg. of MAP for 18 days inhibited heat but upon withdrawal the conception rate and litter size were below the level of untreated controls. Follicles developed to large size but failed to ovulate in 30% of gilts. Increasing the daily dose of MAP to 500 mg. and shortening the treatment period to 9 days, followed by HCG, permitted precise control over ovulation time but incidence of heat and fertility was low. DES given before insemination increased the fertility level of eggs but adversely affected embryo survival. HCG caused ovulation when given to proestrous gilts.

EE given orally to normally cycling gilts for 15 days inhibited heat for 40 days by presumably maintaining the corpora lutea when started on the 1st through the 13th day of the estrous cycle. When started on the 15th through the 19th day of the estrous cycle, the next heat occurred as expected but the second heat was delayed for at least 40 days. Prepuberal gilts given EE for 5 days showed heat and ovulated near the fifth day of treatment. A single subcutaneous injection of 500 I.U. of a PMS-HCG mixture to prepuberal gilts caused heat and ovulation about 5 days later. Neither treatment of prepuberal gilts caused onset of normally recurring estrous cycles.

Literature Cited

(1) Baker, L. N., Ulberg, L. C., Grummer, R. H., and Casida, L. E.
1954. Inhibition of Heat by Progesterone and Its Effect on Subsequent Fertility in Gilts. Jour. Anim. Sci. 13:648.

(2) Braden, A. W. H., Lamond, D. R., and Radford, H. M.
1960. The Control of the Time of Ovulation in Sheep. Austral. Jour. Agr. Res. 11:389.

(3) Brunner, M. A., Hansel, W., and Hogue, D. E.
1964. Use of 6-Methyl-17-Acetoxyprogesterone and Pregnant Mare Serum to Induce and Synchronize Estrus in Ewes. Jour. Anim. Sci. 23:32.

(4) Dauzier, L.
1958. Le controle de la date de la reproduction chez la brebis et al vache. Ann. de Zootech., No. 4, p. 341.

(5) Davies, H. Lloyd.
1960. Reduced Fertility Associated With the Use of Multiple Injections of Progesterone Followed by Pregnant Mare Serum. Austral. Vet. Jour. 36: 20.

(6) Dziuk, P. J. and Baker, R. D.
1962. Induction and Control of Ovulation in Swine. Jour. Anim. Sci. 21:697.

(7) Dziuk, P. J. and Polge, C.
1962. Fertility in Swine After Induced Ovulation. Jour. Reprod. Fert. 4:207.

(8) Dziuk, P. J., Hinds, F. C., Mansfield, M. E., and Baker, R. D.
1964. Follicle Growth and Control of Ovulation in the Ewe Following Treatment With 6-Methyl-17-Acetoxyprogesterone. Jour. Anim. Sci. (to be published).

(9) Evans, J. S., Dutt, R. H. and Simpson, E. C.
1962. Breeding Performance in Ewes After Synchronizing Estrus by Feeding 6-Methyl-17-Acetoxyprogesterone. Jour. Anim. Sci. 21:804.

(10) First, N. L., Stratman, F. W., Rigor, E. M., and Casida, L. E.
1963. Factors Affecting Ovulation and Follicular Cyst Formation in Sows and Gilts Fed 6-Methyl-17-Acetoxyprogesterone. Jour. Anim. Sci. 22:66.

(11) Gardner, M. L., First, N. L. and Casida. L. E.
1963. Effect of Exogenous Estrogens On Corpus Luteum Maintenance in Gilts. Jour. Anim. Sci. 22: 132.

(12) Gerrits, R. J., Fahning, M. L., Meade, R. J. and Graham, E. F.
1962. Effect of Synchronization of Estrus on Fertility in Gilts. Jour. Anim. Sci. 21:1022. (Abst.)

(13) Gordon, I.
1958. The Use of Progesterone and Serum Gonadotrophin (PMS) in the Control of Fertility in Sheep. II. Studies in the Extra-Seasonal Production of Lambs. Jour. Agr. Sci. 50:152.

(14) Gordon, I.
1963. The Induction of Pregnancy in the Anoestrous Ewe by Hormonal Therapy I-V. Jour. Agr. Sci. 60:31.

(15) Hinds, F. C., Dziuk, P. J. and Lewis, J. M.
1964. Control of Estrus and Lambing Performance in Cycling Ewes Fed 6-Methyl-17-Acetoxyprogesterone. Jour. Anim. Sci. (to be published).

(16) Lamond, D. R.
1962. Oestrus and Ovulation Following Administration of Placental Gonadotrophins to Merino Ewes. Austral. Jour. Agr. Res. 13:707.

(17) Lamond, D. R. and Bindon, B. M.
1962. Oestrus, Ovulation, and Fertility Following Suppression of Ovarian Cycles in Merino Ewes by Progesterone. Jour. Reprod. Fert. 4: 57.

(18) Nellor, J. E.
1960. Control of Estrus and Ovulation in Gilts by Orally Effective Compounds. Jour. Anim. Sci. 19:412.

(19) Nellor, J. E., Ahrenhold, J. E., First, N. L. and Hoefer, J. A.
1961. Estrus, Ovulation and Fertility in Gilts Subsequent to the Oral Administration of 6-Methyl-17-Acetoxyprogesterone. Jour. Anim. Sci. 20:22.

(20) Robinson, T. J.
1961. The Time of Ovulation and Efficiency of Fertilization Following Progesterone and Pregnant Mare Serum Treatment in the Cyclic Ewe. Jour. Agr. Sci. 57:129.

Table 1.--Heat and ovulation relative to last MAP in ewes[a]

Last MAP to killing	Total ewes	Ewes in heat	Ewes ovulating
hr.	no.	no.	no.
24	8	0	0
48	8	0	0
60	8	7	0
72	9	4	0
76	10	10	3
78	10	9	5
80	10	6	2
82	9	8	5
84	10	8	5
86	10	9	4
90	9	7	6
94	10	9	6

[a] Modified from Dziuk _et al_., 1964.

Table 2.--Effect of PMS and time of administration on heat and fertility in ewes

Flock	Treatment	Time of PMS to last MAP	Total ewes	Ewes in heat	Lambed to first heat	Lambing rate[a]
		hr.	no.	no.	no.	
A	PMS	0	10	7	1	1.00
A	PMS	-24	10	9	6	1.50
B	PMS	-48	11	11	6	1.50
B	PMS	-24	11	8	7	1.28
C[b]	PMS	-48	18	12	5	1.33
C[b]	PMS	-24	17	11	6	1.66
C[b]	PMS	0	18	9	2	1.00
D	PMS	+24	39	37	28	1.43
D	None	--	29	27	13	1.15
E	PMS	0	24	23	10	1.60
E	None	--	78	74	35	1.31
F[b]	PMS	0	17	12	4	1.75
F[b]	None	--	25	19	9	1.33

[a] Lambs born per ewe lambing.
[b] Anestrous ewes.

No statistically significant differences between treatments in the proportion of ewes showing heat, the conception rate, nor the lambing rate.

Table 3.--Effect of HCG and ECP on heat and fertility in ewes

Flock	Treatment	Dose	Route	Time relative to last MAP	Total ewes	Ewes in heat	Lambed to first heat
		IU		hr.	no.	no.	no.
A	HCG[e] + PMS	500	SQ[a]	-24	10	7	2
B	HCG + PMS	500	SQ	-24	43	39	20
C	HCG + PMS	500	SQ	-24	10	6	2
D	HCG + PMS	500	SQ	0	10	7	3
E	HCG + PMS	500	SQ	0	10	7	4
F	HCG	500	IM[b]	+48	10	5	3
F	HCG	500	IM	+72	10	7	4
F	ECP	2[c]	IV[d]	+48	10	10	2
F	ECP	2[c]	IV	+72	10	10	3

[a] Subcutaneously.
[b] Intramuscularly.
[c] Mg.
[d] Intravenously.
[e] 250 IU PMS plus 250 IU HCG.

No statistically significant differences between flocks or treatments.

Table 4.--Fertility in swine after treatment with MAP

Herd	Treatment	Total gilts	Gilts in heat[a]	Farrowed			Mean pigs born per pregnant sow
				To first mating	To second cycle	Total	
		no.	no.	no.	no.	no.	no.
A	MAP	12	6	6	3	9	9.8
A	None	13	-	7	4	11	10.3
B	MAP	10	12	11	1	12	8.2
B	None	11	-	9	-	9	11.0
C	MAP	29	15	Not[b]	28	11	8.8
C	None	28	-	-	-	17	9.7
D	MAP	13	12	10	2	12	11.4
D	None	17	-	-	-	15	10.5
E	MAP	10	8	5	-	5	11.2
E	None	20	-	-	-	15	11.5
F	MAP	8	1	1	-	1	8.0
F	None	7	-	-	-	3	8.7
G	MAP	19	14	11	2	13	10.3
G	None	18	-	-	-	16	11.5
H	MAP	20	17	Not	15	15	9.2
H	None	15	-	-	-	14	10.5
I	MAP	59	36	36	13	59	9.0
I	None	3	-	-	-	3	9.3
J	MAP	20	-	Not	13	13	7.2
J	None	16	-	-	-	9	10.0
K	MAP	12	10	Not	6	6	8.4
K	None	8	-	-	-	8	8.5
L	MAP	8	6	Not	6	5	9.0
L	None	10	-	-	-	10	9.2
M	MAP	9	8	8	-	8	9.0
M	None	17	-	-	-	13	8.6
N	MAP	20	2	2	5	7	10.1
N	None	4	-	-	-	3	11.6
Total	MAP	258	66%			71%**	9.2**
	None	187				78%	10.2

[a] Gilts in heat days 4-8 after last MAP.

[b] Not permitted to mate.

** MAP inferior to None ($P < 0.01$).

Table 5.--Response of prepuberal gilts to a single subcutaneous injection of gonadotrophin

Dose	Total gilts	Gilts in heat[b]	Gilts ovulating	Corpora lutea per gilt ovulating
IU[a]	no.	no.	%	no.
250	35	18	67	14
250	22	9	?	?
500	83	36	?	?
500	40	18	75	17

[a] Equal proportions of PMS and HCG.
[b] Gilts in heat days 4-6 after injection.

Dr. CASIDA: There is in the audience a potential speaker who was approached earlier about presenting a paper. For some reason he was not inclined to prepare a paper but he did indicate his willingness to comment upon his work and in fact give us a look at it. I am going to call upon Dr. Wiltbank to tell of some of the work he has been doing at Fort Robinson. Jim.

DR. WILTBANK: I want to mention briefly two experiments that we have conducted at Fort Robinson. The first of these we conducted to determine length of heat, time of ovulation, ova transport, fertilization rate and embryonic loss in a group of cycling heifers and a group of synchronized heifers.

We had approximately 50 heifers in each one of these groups. We synchronized the heifers by feeding them 500 mg. of a product from E. H. Squibb and Company called proxone, an acetophenone derivative of 16, 17 dihydroxy-progesterone. This was individually-fed daily. We checked heat on these heifers at four-hour intervals over the length of the experimental period. Ovulation was determined by rectal examination. We examined the heifers at the beginning of heat, twelve hours after the start of heat when they were bred, and then, as soon as estrus ended, we examined them every four hours until ovulation occurred. Forty-eight hours after ovulation, egg recoveries were attempted in half the heifers in each group. We did this by a high lumbar laparotomy. We removed the oviduct and approximately two inches of the uterine horn. Then we cut the oviduct into thirds and flushed each third separately and examined for the presence of the ova.

Now let me show briefly some of the data we have. This is the length of heat in the two groups of animals. (Slides were here projected onto a screen.) The average in the cycling animals was 22 hours and in the synchronized heifers 16-1/2 hours. Most of the cyling heifers stayed in heat longer than 20 hours. Our synchronization process definitely affected the length of heat with this particular compound.

When we timed ovulation from the start of heat, little or no effect was noted on the time that the heifers ovulated. The average length in both groups was 33 hours. There was an effect on the length of time from the end of heat until ovulation. This averaged 11 hours for cycling animals and approximately 17 hours in the synchronized animals.

Now, let me show the egg recovery and the fertilization rate on these two groups of animals. The percentage of eggs recovered was low, 64 percent in each group. We feel that this is because of the frequent palpations near the time of ovulation. We had some other heifers that we were recovering eggs from at approximately the same time as these in which our egg recovery was 85 percent. The numbers of eggs with a broken zona pellucida were one in the cycling and three in synchronized animals. The number of normal eggs recovered was 13 in each group. Preparation of normal eggs fertilized was 12 of 13 in the cycling, and 9 of the 13 in the synchronized heifers. Percent of fertilization in recovered eggs was 86 percent in the cycling heifers and 56 percent in the synchronized group. There was a difference in fertilization in favor of the cycling animals and perhaps a few more eggs that had a broken zona pellucida in the synchronized animals.

Now, as far as ova transport is concerned, the location of the eggs is shown here. (Another slide was projected on a screen.) The total eggs recovered was 14 in the cycling and 16 in the synchronized. Eggs in section 1--this is a section up closest to the infundibulum--one in the cycling and one in the synchronized. Eggs in section 2: 11 eggs in each case. Eggs in section 3: 2 in the cycling and 4 in the synchronized. These data show very little effect on rate of ova transport.

Now, there is a bad thing about these data. The fertilization rate results are not confirmed by the 34 day pregnancy data obtained from the other half of the heifers. We had 26 percent of the cycling heifers that we found pregnant at 34 days versus 54 percent in the synchronized heifers. So what we found in fertilization was just the reverse of what we found in pregnancy diagnosis.

We are repeating this particular project at the present time and we are getting our ovulation data separate from our fertilization data, running two cycles on another group of heifers. The data have not been completed yet.

Now there is one other thing that I would like to mention while I am up here. I differ with Dr. Casida and Dr. Zimbelman on the fact there is not a method for destruction of the corpus luteum. We have data at Fort Robinson in connection with the cow that shows the injection of estrogen, 5 mg. of estradiol valerate will definitely cause regression of the corpus luteum. We can get it in 90 percent of the cases. So I do believe there is a method for destruction of the corpus luteum.

Now there is a thing that happens when you inject estrogen near the end of the cycle, the 16th or 17th day of the cycle, you can cause the animal to go cystic 80 to 90 percent of the time. So you have this after-effect of these injections near the end of the cycle. But in mid-cycle and during the early part of the cycle, you can destroy the corpus luteum and the animal will come back into heat earlier than you would expect it to.

There is another thing I wanted to mention this morning on synchronization of heat that I think it is important. Dr. Zimbelman mentioned that we have a hyperestrogenic effect when we feed MAP. We have evidence at Fort Robinson that when we inject progesterone plus estrogen, we can decrease our dose of progesterone tremendously and still get synchronization. We have gone down to as low as 10mg. of progesterone daily when we add 160 micrograms of estrogen and we got very good synchronization. In fact, we can induce synchronization with as short an injection period as 10-12 days, which would indicate that perhaps we are getting some regression of the corpus luteum and then be able to synchronize these animals.

DR. CASIDA: Thank you, Dr. Wiltbank.

While you are getting your questions formulated, I would like to raise a question or two with my colleagues.

Dr. Dzuik, did you intimate that there may be a different set of ram differences in fertility when they are used on synchronized ewes that when they are used on normal cycling ewes? In other words, ram A and B may differ from each other when used on synchronized ewes, but the spread between them will differ when they are used on normal ewes.

DR. DZIUK: Yes, this is a part of the problem because a ram may mate one ewe or two ewes in heat today, several times, and finally settle them, whereas, if he has an allotment of eight ewes and all of them are in heat, he mates at least once or twice with each ewe so we know that mating has taken place. This may not be the same as turning them loose with one or two in one day. I am going to hedge on the question because I think it may be related to the capacity of the ram that we cannot measure when he is turned loose, or just by single semen evaluation of any kind. He may appear to be perfectly fertile and not be fertile under the conditions, where eight or ten are in heat in one day.

DR. CASIDA: Thank you, Dr. Dziuk. I raised the question on the possibility of bringing out differences between ram sperm when they are subjected to the environment of post-synchronization as compared to cycling. Now, your difference between return ewes at the second heat and the first heat is actually following the synchronization compared to no synchronization, is that not right?

DR. DZIUK: Yes. That is true in most cases. We thought the second heat after synchronization was less fertile than the first, so we grouped the animals so their first heat coincided exactly in terms of the day of the calendar as the second heat of others, and we still got all these differences, and we didn't get any differences between the treatments. The first cycle seemed to be as fertile as the second one when we took the ram differences and the season differences.

DR. CASIDA: I would like to raise one question with Dr. Wiltbank. He has referred to the regression of corpora lutea when he has used estrogen. I would like to ask: Is this the regression of a fully formed corpus, or is it the prevention of the formation of the young corpus?

DR. WILTBANK: It is a regression of the fully formed corpus luteum because we have injected heifers at the 7th and 10th day and the corpus luteum regresses. And if you inject heifers the 3rd or 4th day of the cycle, the corpus luteum will go ahead and you can palpate it by rectal examination to be 18 to 20mm., and then it will start regressing. So it is not prevention of formation, but regression.

DR. CASIDA: I raised that question because there is evidence of differences in usage of the term "luteinizing," and "luteotropin," or "luteotropic" action. We may need to distinguish between those things which bring about the formation of a normal corpus from those things which maintain the structure and function of a normal corpus.

I would like to raise the question with Dr. Zimbelman from this morning's discussion with regard to this post-partum cows he is treating with MAP and in which there appears to be an acceleration of ovarian activity in terms of the first post-treatment ovulation. Is this actually an acceleration of activity or is it the prevention of what may have been quiet ovulations in untreated animals so that corpora lutea did not have to be "cleared out" of the ovary before they came back into estrus after treatment?

DR. ZIMBELMAN: I am not sure that I have the question completely straight, but let me start here. Our initial hope was that we would have cows which would not ovulate until 45 or 50 days post-partum, in which case we would have had a distinct shortening of the interval. As you can see, our intervals to first post-partum ovulation were 37 days or less, and therefore we were not able to really shorten it as much as we had hoped. I think that all we can say is that we synchronized these cows by starting them on treatment prior to the time that they began normal estrual cycles, but we cannot say that we really initiated them before because, as you know, some of our controls did ovulate during treatment, whereas the treated animals did not. So, in a sense, there were many animals which we only delayed as you would an already-cycling animal until the end of treatment, but, if this had been the only thing, I think you would expect an average interval from last feeding for control animals of about 11 days, based on chance alone.

If you treated cows that were coming in on each of 21 days on chance alone, you would have the average interval of untreated animals of 10-1/2 days, and over average was about 8 days, so this would indicate that perhaps some animals were being speeded up by a few days.

Is that reasoning clear?

DR. CASIDA: Thank you, Bob. I raised this question in part because I agree with you thoroughly on the difficulty of palpating the post-partum ovary in a beef cow, and the frequent inaccuracy or inability to pick up corpora lutea at that stage, so it would seem to me that this quiet ovulation might have been a factor in this situation.

DR. ZIMBELMAN: I would like to ask Dr. Wiltbank how much variation there is in the interval from the injection of estradiol valerate to the regression of corpora lutea in animals of different stages of this cycle?

DR. WILTBANK: The corpora lutea will regress anywhere from 4 to about 10 days after the injection of the estradiol valerate, so that it is not a very consistent time.

DR. CLARENCE HULET, USDA, DuBois, Idaho: This is not a question, but I think that I have an answer to Dr. Dziuk's question.

We designed a synchronization experiment in which we fed MAP to ewes in such a way that both those which had been synchronized subsequently would come in heat at the same time; the first group having their post-treatment estrus simultaneously with another group having their second post-treatment estrus. We still got this difference in fertility. In other words, of those which had their first post-treatment estrus but which were mated at the same time as those which were simultaneously having their second post-treatment estrus, 60 percent lambed to that mating, whereas 80 percent of those which had their second post-treatment estrus lambed to simultaneous matings.

DR. CASIDA: Thank you, Dr. Hulet.

Now there has been a question handed in which I will ask Dr. Dziuk to answer. Why is ECP fatal interarterially and not intravenously?

DR: DZIUK: I presume that the artery is carrying this oily solution directly to the brain and, probably crudely stated, "clogging" up the works. And intravenously it apparently makes a circle around the body and, if it does do damage, it does it in such scattered areas that it does not do any real harm. A suggestion here that it could be due to hemorrhage is not very likely, because intraarterial injection of these oily solutions has an immediate effect, in something like five seconds.

DR. DHINDSA from Illinois: Is death due to the ECP or the injection of the oil into the artery?

DR. DZIUK: The only injections we made were ECP and oil, so that I cannot say that I have separated them out, but my guess is that it is the oil itself.

DR. CASIDA: I would like to ask Dr. Hansel to come forward and take care of a question growing out of this morning's discussion.

DR. HANSEL: Thank you, Dr. Casida. This is asked by Dr. Dhindsa, and I am not sure that I can really answer this. It is:

What is your definition of cystic follicles insofar as size, color, etc., in pigs and in cattle?

This is not an easy question to answer offhand as most of you know. There are certain histological abnormalities that accompany cysts, such as luteinization of theca interna. I doubt that there is any arbitrary size that one can place on this, say beyond this size it is a cyst, and below this it is not. It is something I would be glad to discuss with you. Some of the more detailed observations one can see histologically, but I do not want to get into a long discussion of them now.

DR. CASIDA: I was thinking that Cornell University had given the classical definition of a cyst, that it had to be a structure in the cow that was more than 25mm. in diameter.

DR. SORENSON, Texas A. & M: I would like to make on comment first and then ask a question.

The comment concerned first and second estrus breeding. We put a group of Santa Gertudis heifers on an experiment in which we bred 52 of these heifers at the first synchronized estrus, skipping first estrus on an additional 52 and breeding at second estrus. And then we had a third group of 52 heifers that were controls.

The first estrus group, that is, those breed at their first estrus, had 25 percent conception.

The second estrus group, the one that we skipped the first estrus, had 34.6 percent conception.

If you want to make comparisons between the second breedings, the group that we bred the first estrus and them followed up with a second breeding also, we got 40 percent conception. So this sort of "blew" out idea that if we skipped this first estrus, we would get most of them at the second estrus. We actually got more in the ones that we bred at first estrus, picking up at the second estrus, than we did where we skipped the first estrus.

In our control animals, at first estrus we got 54 percent conception, followed with a 17 percent conception on second estrus. So I am still concerned about our second estrus. I am not sure that we can skip this first one and expect to pick up as many as we thought on second estrus.

Now I have a question.

One of the side-effects that we have noticed in cattle breeding, following our synchronization of estrus, has been the copious mucus secretion. This was mentioned just briefly this morning, but no comment was made on it.

We have found, especially in some of our crossbred cattle, that as much as 500 cc of mucus may be expelled from the vagina at the time of breeding. This is when the animals are in estrus two to five days following the last feeding. This has been both with MAP and with CAP.

Our last trial with MAP was one I think that was most critical. We had a group of Brangus cows. These cows had calves by their side and were fed for an 18-day period, 180 mg. per day level. There was very much mucus present at the time of breeding, and I would like for some comment here to be made on what effect this may have upon our conception rates.

DR. CASIDA: I think I shall ask Dr. Zimbelman if he has any further comment on this question.

DR. ZIMBELMAN: If you will recall, the animals that I showed you this morning in Trial A that had received 100 and 500 mg. of MAP as a single injection and slaughtered at 126 days after injection, the animals on 500 mg. had a highly significant increase in uterine weight.

On gross observation, I would say this was primarily due to an accumulation of mucus, primarily cervical mucus. Some of the uterine horns did contain mucus, but this was very tenacious.

It wasn't at all fluid like as I think Dr. Dutt described the accumulation which he had noted in ewes.

This was not present in the 100-mg. MAP group. But most of these animals had returned to ovulation and therefore had perhaps experienced an opportunity to pass this mucus.

Based on these data alone, we cannot differentiate between the possibility of it not being produced by 100-mg. dose, in contrast to the possibility that the mucus had a chance to pass by the animal having experienced one estrous cycle.

Based on some other observations, I think probably the latter choice is correct and that is, if we use oral administration, we have not observed much increase in mucus, particularly on short-term treatments as Dr. Sorenson is referring to. I have no explanation, no prior observations that are similar to those.

We do have some longer-term treatments with oral progestogens in which we have a slight accumulation of mucus, but if the animals were killed at 8 days after treatment, the uterine weights were actually reduced, indicating that the mucus was contributing primarily to the uterine weight increase.

DR. CASIDA: Thank you, Dr. Zimbelman. I shall ask Dr. Wiltbank to make a comment in this regard.

DR. WILTBANK: We have not seen an increase in mucus in our synchronized animals. We have done most of our work with the product of Squibb, although we have done some with MAP, and we never noticed the increase in mucus there.

DR. CASIDA: Thank you, Dr. Wiltbank. Are there other comments to this question or this point of Dr. Sorenson's?

DR. WAGNER of Eli Lilly: In reference to the mucus situation, I would not want to leave anyone with the impression that MAP is the only progestogen that causes copious mucus flow.

We have seen this almost 100 percent of the time, not in 100 percent of the animals. In 100 percent of the studies we have conducted, both at Greenfield and other places, we saw this mucus in 50 to 60 percent of the animals. It seems to be more prevalent in those animals which are started on treatment toward the middle of the cycle where they have an extended follicular phase throughout the progestational period.

I think possibly Dr. Zimbelman's comment that 10 mg. is a little high in cattle is correct. Upon Dr. Hansel's suggestion, we reduced this 10 mg. dosage of CAP the latter half of the feeding period, nine days, to 5 mg. and the mucus was less than normal at the heat period. We had I think 28 animals. Twenty-six showed heat within a 36-hour period and actually, upon insemination, only two or three showed any mucus discharge at all. In all respects, vulvar swelling, mucus discharge, the intensity of the heat, appeared to be more normal, with the exception of mucus being a little less than normal.

This is very recent and we have only had a chance to look at it once. I think it does deserve comment because mucus has been brought up in the discussion as an abnormal function in this first synchronized heat following progestin treatment.

DR. CASIDA: Thank you, Dr. Wagner. I am not sure that anyone has left any implication as to whether mucus is a desirable or an undesirable side-effect.

We have another question which has been handed to Dr. Dziuk to answer.

DR. DZIUK: The question is:

How did you establish that the fertility failures you have reported associated with MAP treatment were due to impaired sperm transport?

First of Wisconsin takes credit for the question and he wants me to take credit for the answer, I guess.

The treatments that we used were high levels of progesterone followed by HCG, then flushing the eggs out of the oviduct and noticing if they were fertilized and if sperm were present. The eggs appeared to be perfectly normal and, if the animals showed heat, that is, they had enough endogenous estrogen, then we got fertility. And when we gave estrogen, we did get sperm transport and fertility.

We also gave estrogen to pigs that we did not give HCG to, so there was no ovulation and no eggs there, and we could recover sperm. However, we could never recover sperm from those animals receiving HCG which did not show heat and we did not get fertilized eggs up there. We did not get sperm in the oviduct. Now we got motile sperm in the uterus as long as 48 hours after insemination in these pigs that ovulated but did not show heat. We found that when the eggs went down into the uterus, they could pick up the sperm down in the uterus even 48 hours after they had been ovulated, but, of course, then it is too late as far as the egg is concerned. We gave the HCG, no estrogen, and we got eggs, no sperm, no sperm in the oviduct, no sperm on the eggs. Then we gave estrogen and we got sperm on the eggs and reasonable fertility. Then we gave the estrogen without the HCG, so the estrogen is there, and we got sperm in the oviduct with no eggs. So that maybe the estrogen in some way enhances the capacitation as Dr. Casida mentioned, but my guess is that it is probably pretty simply transport, at least to start with. They have to be there anyway whether they are capacitated or not.

Have you taken control animals and treated animals and attempted to recover or quantitate the recovery of sperm from the oviducts in both so that you know pretty definitely that they are or are not being transported?

DR. DZIUK: Note these eggs were recovered 14-24 hours after ovulation at which time they should have been fertilized and should have a reasonable number of sperm on the zona pellucida. You do not find any sperm and, in these animals in which we did not get fertility, there are no sperm there at all. And none in the oviduct that we could recover by any means.

DR. FIRST: I still raise the question with you in view of the difficulty of sperm recovery from the oviduct that, because you do not find the sperm in the egg, does not necessarily mean that they were not in the oviduct.

DR. DZIUK: No, not necessarily, but when we can give estrogen and get sperm in the oviduct and sperm on the eggs, then there is at least circumstantial evidence, and it satisfies me anyway.

DR. DAVID POPE of British Drug Houses: I would like to know if anybody here has run into a problem where they have been able to synchronize estrus in cycling cattle, but where subsequently the second estrus has not occurred and in which there is a condition in the ovaries where there is persistent corpora lutea, not cystic, but which are adherent to the ovary, which is encased in a thick integument. Unfortunately, I cannot give you histological details. I do not have them yet.

And secondly, of an occasion where animals are synchronized and appear to come into estrus, but will not accept a bull in natural service.

If you could help me, I would be most interested.

DR. CASIDA: I would like to raise a question as to your evidence that these are persistent corpora lutea.

DR. POPE: Unfortunately, my evidence is very slim. This is not my own work. I am just having to repeat what has been told to me. The evidence has been based on intermittent rectal examinations where the corpora lutea could be palpated. The thick integument was also apparent before the animals were destroyed.

DR. CASIDA: Thank you. Have we an answer to this question? Who has had a similar experience of such treatment causing persistent corpora lutea?

DR. HANSEL: We have not had any experience with the persistent corpora lutea. However, I believe the answer to the second part of your question where the animals are synchronized, come in heat, but will not accept the bull, is a matter of waiting a while. We ran into this quite clearly last summer on the beef animals I was describing this morning. They would come in heat and stand for a cow several hours before they would stand for a bull. This is apparently quite normal behavior but I had not realized it before. We would cut them out of the herd when they would stand for cows, put them in with the bull, and several hours would often go by before these animals would breed. It is apparent you just did not wait long enough.

DR. WILTBANK: One other comment on that, in some of our cattle, we found most of them came in heat at 10 'oclock at night. If they are only going to stay in heat eight hours, as some of our synchronized animals did, they would not stand for a bull during the normal time when you are checking for heat.

DR. CARNAHAN of Kansas State: I have noticed, on both a beef herd and on a dairy herd that a portion of the cows that have not conceived after treatment go into anestrus. After a period of three or four months, they then start silent cycling or development of some follicles. A little later some of these cows develop corpora lutea and, after a time, they finally come back into normal cycling and apparently normal conception. I have run into this with numerous cows, both in beef and dairy herds.

DR. CASIDA: Is there the implication here of a delayed effect for perhaps two or three months after treatment?

DR. CARNAHAN: There is an implication that there is a nonbeneficial effect for up to six months. Usually you do not see this anestrus for about two months after removal from treatment. Then I have run into trouble with these cows and have had no success whatsoever with any hormone I have tried.

DR. CASIDA: One further question. Have you seen this in situations of a controlled experiment? So you have a good reason to believe that it is actually more frequent in treated animals than in quite comparable animals that have not been treated, but at the same period of time in life, season and so forth?

DR. CARNAHAN: Yes, I think Dr. Marion can help on this in some of the herds of cattle they had there at K-State. The other, the beef herd that I mentioned, all cattle were treated, so I couldn't. I don't believe he treated all of the cattle all of the time, did you?

DR. CASIDA: Have you a comment, Dr. Marion?

DR. MARION: There is no further comment.

DR. SORENSON: This morning Dr. Hansel made a statement concerning the cattle at the Briarcliff Farm that had calves on them at the time of the start of the experiments. When they were taken off experiment, they started cycling in a short time.

I wanted to ask him if he throught this was due to corpus luteum or was it due to the lack of oxytocin being stimulated by these calves suckling. He mentioned these were crossbred animals. Our experience has been with anything that has Indian breeding in it, you mentioned Charollais, but we do not have any Charollais in the United States, except maybe one or two here and there. They are Charollais, mostly, perhaps, but most of them still have Brahman blood in them. We have found that our Brahman crossbred calves may nurse three and four times as many times a day as our European breeds. And therefore we have a great deal of difficulty in our Gulf Coast regions where we have a preponderance of these crossbred cattle of getting cattle to come back into estrus while they are suckling a calf. The minute the calf is weaned the cattle will be in heat in about a week. But I wanted to pose this question to you: Is this oxytocin or is it the corpus luteum?

DR. HANSEL: Of course, I don't really know. I would certainly like to look into the possibility of oxytocin.

Actually, I do not separate the two effects in my own mind. I do not know how far to go back to start answering this question.

We are fairly well convinced that oxytocin does cause the release of LH now, and we are also fairly well convinced that the LH is luteotropin in the cow, so it could well be failure of the corpus luteum that still involves LH. We will present the data for these rash statements a little later.

DR. DONALDSON Australia via Cornell: I would like to comment on the anestrus in the Brahma crossbred cattle while the calf is suckling.

We have had the same experience in Australia in crossbred and also in purebred cattle that show the British breeds. So I would not like to see the crossbreds implicated as being the sole victims of this phenomenon.

And we feel that nutrition is playing an extremely important part in this suckling effect.

DR. SORENSON: I will agree with that.

DR. DONALDSON: Thank you.

DR. SORENSON: European cattle on poor nutrition won't do it.

DR. CASIDA: Dr. Wagner this morning you mentioned that in attempts to synchronize the post-partum ewe the success with the non-lactating ewes was poorer than with the lactating. Have I indicated that correctly?

DR. WAGNER: Yes and no.

DR. CASIDA: All right. For the moment I am going to accept that you have said yes so I can ask the question. All indications that we have found in cattle and in swine, and to some extent in sheep, are that early weaning may cause various ovarian abnormalities and I believe if you weaned your lambs at four weeks, the time of attempted synchronization was super-imposed on a period of roughly the peak of lactation. Is that correct?

DR. WAGNER: That sounds pretty close.

In reference to this question, I think it should be pointed out, one, that we were surprised at the response we got and that we have had better results in other dry ewes.

This would not be quite the same group of animals because often times they are dry because they have lambed early in the spring and they have been dried for a long time.

In reference to this particular experiment, they were weaned, and again I am guessing, at three to four weeks after lambing. This would vary, of course, in the group, but equal in both groups, and a two-week period followed before the beginning of treatment and then a 16-day period plus a couple of more days before they were in heat, so you have approximately 30 some days after weaning before they are given the first PMS and bred.

Also, my question to you would be whether this abnormality in the polyestrous animal such as the pig and the cow that you see after early weaning, would carry over to the ewe which we would assume is anestrous whether she is lactating or not in the spring?

DR. CASIDA: Fortunately this is the kind of meeting in which we are not expected to answer the questions. We speculate and raise questions for further study.

So thank you, Dr. Wagner.

Well, gentlemen, we have reached the end of this portion of the program. I appreciate very much the way you have helped during this discussion period.

MECHANISMS CONTROLLING THE FORMATION AND PERSISTENCE OF THE CORPUS LUTEUM[1]

L. L. Anderson[2] and R. M. Melampy
Iowa State University, Ames

Mechanisms which control formation of corpora lutea during the estrous cycle, and particularly factors affecting their persistence and regression in various reproductive stages, have been of considerable interest to physiologists concerned with developing methods for control of the estrous cycle in domestic animals. Some recent reviews on control of ovarian function are those by Chester Jones and Ball (30), Anderson et al. (9) and Short (121).

Morphologic Aspects

The mammalian ovary has two principal functions: the production and release of ova and the synthesis and secretion of hormones which regulate the reproductive tract and secondary sexual characteristics. These hormones also influence mating behavior and affect metabolism. Following ovulation, the wall of the ruptured follicle undergoes structural and functional changes which transform it into a transient endocrine gland known as the corpus luteum. While the early development of the corpus lateum appears to be quite similar in many mammalian species, the functional life span varies according to whether the animal is nonpregnant, pseudopregnant, pregnant or lactating. In eutherian mammals, it is generally accepted that the granulosa cells are transformed into luteal cells of the corpus lateum. The fate of theca interna cells is less clear and there appear to be species differences as to their subsequent functional significance in the corpus luteum. The literature pertaining to the histogenesis of the corpus luteum has been reviewed by Marshall (79); Corner (31, 32); Hett (60); Pratt (103); Harrison (58, 59) and Brambell (23). According to Amoroso and Finn (2) the original description of the corpus luteum is usually credited to Volcherus Coiter in 1573, but Harrison (58) has stated that Vesalius had observed it in the human ovary about 30 years earlier.

The developmental morphology of the corpus luteum in the ewe has been described by Marshall (78), Grant (50), Quinlan and Mare (105), Casida and McKenzie (28) and Warbritton (128); in the goat by Harrison (57); in the sow by Corner (31, 32, 34, 35) and Barker (19); in the cow by McNutt (73, 74), Hammond (52), Höfliger (62) and Asdell et al. (17); and in the mare by Harrison (56). The mature bovine corpus luteum may show a fluid-filled cavity, whereas this gland is a solid structure in the ewe, goat and sow. According to Harrison (59), it has been observed in several species that theca interna cells invade the granulosa between day 1 and day 3 and that vascularization of the gland occurs at about the same time. Nearly every cell has an endothelial covering by day 12. The reticulum found between luteal cells is produced by the theca interna according to Solomons and Gatenby (126), but Corner (32, 33) stated that it is probably laid down by endothelial cells. Corner (35) investigated the distribution of the theca interna cells in porcine corpora lutea and found these cells scattered among the granulosa cells at day 18 of gestation. It was difficult, however, to differentiate theca interna cells after this time.

In the sow, Corner (31, 32) observed three principal types of luteal cells in the corpora lutea of pregnancy: (1) true lutein cells originating from the granulosa; (2) cells with smaller round or oval and more chromatic nuclei which appear on the periphery of the gland and along the connective tissue septa and (3) cells with a spindle shape and a cytoplasm which stained dark brown or purple with Mallory's stain. It was also noted that there were transitional stages among the three types.

In evaluating the physiologic aspects of the formation and persistence of the corpus luteum, it is desirable to consider briefly the morphologic development and retrogression of this gland. In the ewe, according to Warbritton (128), the corpus luteum develops from both the granulosa and theca interna, but the luteal cells of the mature gland appear to originate entirely from the former. Three types of cells (embryonic, normal and regressing) were noted and these represented three phases in the life cycle of a single luteal cell derived from the granulosa. The ovine corpus luteum reaches its maximum size at about the middle of the cycle (Casida and McKenzie, 28). The color of the gland changes from blood red in an early corpus luteum through translucent pink, opaque pink, cream and finally yellow.

---

[1] Journal Paper No. J-4910 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1325. Supported by USPHS, National Institutes of Health (Grant HD 01168-05) and American Cyanamid Co., Princeton, N.J.

[2] Lalor Foundation Fellow, 1964, at the Station de Recherches de Physiologie Animale, Centre National de Recherches Zootechniques, Jouy-en-Josas, France.

Corner (34) noted in the sow that, during the week following ovulation, corpora lutea attain a diameter of 8 to 10 mm. If the animal is pregnant, there is further growth until an average diameter of 10 to 11 mm. is reached. Histologically it has not been possible to distinguish between glands of the cycle and those of early pregnance. At approximately day 16 of the cycle, a change occurs in the appearance of the corpus luteum in non-pregnant animals. By day 18 the diameter decreases to 6 mm., and the color changes from pink of active capillary circulation to whitish of scar tissue, indicating retrogressive changes in the nature of fibrous involution. Eventually all that remains of the site of an ovarian follicle, and subsequently a corpus luteum (either of a cycle or pregnancy), is a small mass of scar tissue, a corpus albicans.

McNutt (73, 74) studied the cyclic bovine corpus luteum as well as the corpus luteum of pregnancy and concluded that the luteal cells arise from both the granulosa and theca interna, but he added that many luteal cells exhibited intermediate characteristics and that the origin of these cells could not be stated with certainty. The newly formed corpus luteum may be identified on the fifth day when it protrudes above the level of the ovary. The young corpus luteum measures about 6 to 8 mm. in diameter. By 8 days it has increased to 18 to 20 mm., and when it is mature it measures 20 to 25 mm. The cyclic corpus luteum begins to regress about day 16 following estrus. There is, however, no marked reduction in size until the organ is 18 to 20 days old.

Melampy and Gay (83) made a study of the weight of both the ovary and its corpus luteum of pregnancy in the cow. The estimated age of the corpus luteum was based on the crown-rump length of the fetus. In the 298 cows examined, 90 percent of the ovaries, including the corpus luteum of pregnancy, weighed between 6 and 13 gm. Luteal weights ranged from 3.0 to 6.5 gm. and were approximately one-half of the total ovarian weight. No relationship was observed between the weight of the ovary or the corpus luteum and the stage of pregnancy in the cow. The mean luteal weights in grams were as follows: Angus 4.8, Hereford 4.7, Shorthorn 5.2 and Holstein 5.4. The mean weights for each breed were not significantly different.

According to Asdell (16) the corpus luteum becomes slightly larger during pregnancy than it is in the cycle, and the greatest size is attained at about 4 months, after which time the size and weight remain about constant. Retrogressive changes begin a little before parturition. According to Boyd (21) the time of its disappearance is from 30 to 90 days after calving, but occasionally it disappears earlier than 30 days.

Results of histological and histochemical investigations of the bovine corpus luteum have been reported by Weeth and Herman (129) and Foley and Reece (48). The latter investigated the gross and microscopic anatomy of the bovine corpus luteum between 25 and 45 days of gestation and stressed the variation in size, shape and staining qualities of the individual luteal cells. It was demonstrated that the luteal tissue was more compact and that the cells were larger and more rounding in shape, with the cytoplasm being more lightly stained between days 25 and 30. Moss et al. (95, 96) have reported studies dealing with the histochemistry of the bovine reproductive tract, including the corpus luteum. These investigators noted that the cyclic corpus luteum contained large amounts of alkaline phosphatase until about the thirteenth day of the cycle. This enzyme was very low or, except for capillary endothelium, absent in later stages of the cycle. Both theca and granulosa luteal cells contain phosphatase activity up to mid-cycle; it is first lost from the granulosa cells and later from the theca cells. The corpus luteum of cycling cows differs markedly from the corpus luteum of pregnancy with regard to the presence of phosphatase. The presence of phosphatase in the former and not in the latter would suggest that phosphatase is not concerned in the secretory activity of the corpus luteum but, rather, may be concerned in the initial stages of growth and development of this organ.

The luteal cells of bovine corpora lutea between 16 and 33 days of gestation have been classified into five types on the basis of their cytological characteristics by Foley and Greenstein (47). Type I cells represent "immature" luteal cells and Type II are mature cells which have reached their maximum size and development. Type III cells are believed to be in the initial stage of regression which continues through Type IV and terminates with Type V cells. Between 16 and 33 days of gestation, there is an increase in the number and the size of the Type II cells with a corresponding reduction in the Type I. These investigators concluded that the cytological changes in the bovine corpus luteum during early pregnancy coincide with advancing gestation and are related to the reproductive performance of the individual animal.

Physiologic Aspects

Stimulation of ovulable follicles in rats with luteinizing hormone (LH) causes the formation of corpora lutea, and the maintenance of these structures in a functional capacity is thought to be due to pituitary luteotropin, which is apparently identical with prolactin (Astwood, 18; Evans et al., 44, 45). Evidence for a pituitary luteotropin in the rat is particularly well demonstrated by maintenance of corpora lutea, but not follicles, for several months following autotransplantation of the pituitary to the kidney (Everett, 46). Intact rats with a pituitary homotransplanted beneath the kidney capsule show a pseudopregnancy-like prolongation of the diestrum in progress at the time of transplantation, with a tendency for subsequent cycles to be prolonged (Quilligan and Rothchild, 104). The stimulus of mating is necessary for pseudopregnancy in the spontaneously ovulating rat and mouse. Pseudopregnancy follows ovulation with or without mating in induced ovulators, such as the rabbit and ferret. Corpora lutea capable of secreting progesterone form during non-fertile cycles in spontaneously ovulating domestic animals, such as the cow, sow, and ewe. Prolactin alone is unable to provide luteotropic effects in the rabbit (Hilliard et al., 61; Kilpatrick et al., 68, 69; Rennie et al., 107); guinea pig (Aldred et al., 1; Rowlands, 115); sow (Duncan et al., 43; Sammelwitz and Nalbandov, 117); ewe (Denamur and Mauléon, 40; Moore and Nalbandov, 94); cow (Smith et al., 125) or woman (Bradbury et al., 22; Holmstrom and Jones, 63).

Rothchild (110) reported that lactation is maintained in oxytocin-treated hypophysectomized rats bearing pituitary autotransplants; thus, luteotropin (LTH) secretion does not depend on stimulation from the central nervous system (CNS). It was proposed that a CNS influence inhibits LTH secretion and that another CNS influence stimulates FSH and LH secretion; therefore, progesterone could maintain LTH secretion through its ability to suppress the CNS inhibition over LTH. A decrease in progesterone secretion allows the CNS inhibitor to increase its activity, thereby resulting in decreased LTH secretion. As a result, progesterone secretion is reduced to a lower level leading to complete cessation of both LTH and progesterone secretion and consequently to the regression of the corpus luteum. Progesterone injected into rats throughout the entire pregnancy or pseudopregnancy does not alter luteal function (Sammelwitz et al., 116). The same lack of effect of treatment with progesterone on the size of the corpora lutea was observed in hypophysectomized rats with autotransplanted pituitaries. These findings demonstrate that progesterone does not inhibit the secretion of pituitary luteotropin in the rat (Rothchild, 111). Increased levels of progesterone may prolong the life of corpora lutea in the rat by maintaining secretion of a pituitary luteotropin (prolactin, LTH) (de Jongh and Wolthuis, 66; Rothchild, 112). Furthermore, the effect of hysterectomy in this species may be due to a deficiency or diminished secretion of LH, thus decreasing the luteolytic effectiveness of the pituitary gland. Estrogen treatment alone or combined with progesterone, depressed LH secretion in the rat, but progesterone treatment alone did not depress secretion of LH (Rothchild, 113). When pituitary transplantation was combined with progesterone treatment, luteolysis occurred, but when pituitary transplantation was combined with estrogen treatment luteolysis usually did not result. Maintenance of prolactin secretion in the rat may depend upon progesterone and the failure of prolactin secretion may result from the depressive effect of LH upon progesterone secretion (Rothchild, 113). Thus, prolactin secretion and absence of LH appear necessary for persistence of the rat corpus luteum, and this will occur for prolonged periods when the pituitary is free of inhibitory affects of the CNS on prolactin secretion.

In 1961, Nalbandov proposed that the discharge of a pituitary luteotropin (defined as an unidentified substance or possibly LH) over a relatively short time (no longer than 2 to 3 days in guinea pigs and pigs) is sufficient to maintain corpora lutea for their normal life span during the estrous cycle. Furthermore, no further release of pituitary luteotropin occurs unless the female becomes pregnant, and, in this case, intrauterine events of implantation cause a secondary release of the pituitary luteotropin which may then be continuous throughout the gestation. These hypotheses were based on the assumption that the luteolytic effect of exogenous progesterone is not due to a direct action on progesterone of the formed corpora lutea and that sufficient levels of exogenous progesterone block secretion of a pituitary luteotropic substance. Sammelwitz et al. (116) demonstrated that high doses of progesterone injected into pregnant pigs from the time of ovulation until days 10 to 13 of gestation did not prevent formation of corpora lutea, whereas progesterone injections begun on days 12 to 16 of pregnancy resulted in complete and rapid destruction of the formed corpora lutea. Recently, Brinkley et al. (25) reported that exogenous progesterone beginning 1 or 2 days before ovulation, the day of ovulation, or 1 day after ovulation could not prevent the formation and maintenance of corpora lutea during the normal duration of the luteal phase of the cycle. Corpora lutea in these progesterone-treated gilts were normal as indicated by chemical determination of progesterone content of the tissue at slaughter. From these observations in the pig (Brinkley et al., 25), it was concluded that the pituitary luteotropin needed for corpus luteum maintenance is released either before or simultaneously with the release of LH and that this hormone is not required beyond the initial "impetus" for formation of functional corpora lutea. Du Mesnil du Buisson and Léglise (88) found that corpora lutea formed in pigs hypophysectomized only a few hours after the first signs of estrus. Also, corpora lutea were morphologically normal, contained normal concentrations of progesterone, but were generally smaller at days 13 and 14 of the cycle when hypophysectomies were performed during early luteal phases of the cycle. These results tend to support the contention that a pituitary luteotropin secreted during the initial phases of the estrous cycle may be sufficient for the formation and maintenance of the corpora lutea of that cycle. In progestin-treated nonpregnant gilts with follicular growth induced by pregnant mare serum (PMS) treatment, a single injection of an ovulatory-dose of human chorionic ganadotropin (HCG) was sufficient to cause ovulation and formation of normal corpora lutea, as indicated by progesterone content (Brinkley et al., 24). However, daily injections of HCG in progestin-blocked pregnant pigs will support morphologically normal corpora lutea for as long as 16 days, but the luteal tissue is not physiologically normal as shown by reduced progesterone concentrations. Thus, HCG apparently is without luteotropic action in the pregnant animal. It was concluded that corpora lutea of pregnancy, unlike those corpora lutea of the estrous cycle, are supported by a continuous or sustained release of a luteotropic substance.

Using prepubertal lambs, Denamur and Mauléon (40) found both that 1 to 3 ovulations could be induced with injections of PMS and chorionic gonadotropin and that corpora lutea formed were morphologically and histologically normal. These corpora lutea remained functional until 12 days post-ovulation; the onset of luteal regression occurred between the 12th and the 16th days, and regression was complete by the 20th day. Similar development, maintenance and regression of corpora lutea occurred in treated lambs hypophysectomized on the day of induced ovulation.

Hypophysectomy or section of the pituitary stalk does not terminate pregnancy in ewes when these operations are performed at various stages from the 42nd to 90th days of gestation (Cowie et al., 36; Denamur and Martinet, 38). Gestation also continues when ovariectomy precedes hypophysectomy at mid-gestation (Denamur and Martinet, 38). In goats, however, hypophysectomy or pituitary stalk section is followed by abortion within a few days (Cowie et al., 36). Luteal regression also occurs following hypophysectomy in pseudopregnant ferrets, but stalk section of the pituitary is compatible with full development of corpora lutea at least during the first month of pseudopregnancy (Donovan, 41). The isolated pituitary in this species may store and secrete some luteotropin; however, the corpora lutea eventually regress. It was further suggested that estrogen as well as a pituitary luteotropin may be required for luteal maintenance in the ferret.

Results of investigations have shown that exogenous estrogen influences the luteal function in various species (Amoroso and Finn, 2). Kidder et al. (67) injected gilts with 3 mg. diethylstilbestrol on either the 6th, 11th or 16th day of the cycle and observed cycle lengths of 19, 78, 25, 60 and 18 days respectively. Corpora lutea were maintained until slaughter at 34 days post-estrum in 19 of 20 gilts injected daily with 7.5 or 15 mg. of estrone or estradiol-17β per day beginning on day 11 of the cycle (Gardner et al., 49). Bowerman et al. (20) have investigated the quantities of urinary estrogen metabolites from gilts during the estrous cycle and pregnancy and examined the possibility of a uterine influence on the metabolism of estrogen by comparing urinary metabolites from intact and hysterectomized and from ovariectomized and ovariectomized-hysterectomized gilts following administration of exogenous hormones. Quantities of estrone were low in urine at several stages from hysterectomized animals in comparison to cycling and pregnant animals. Rombauts and du Mesnil du Buisson (109) recently reported that urinary excretion of estrone remained low in sows following hysterectomy on day 70 of gestation. Results from experiments with ovariectomized and ovariectomized-hysterectomized animals did not show any marked influence of the uterus or progesterone-stimulated uterus on the manner in which injected estradiol benzoate was eliminated in the urine (Bowerman et al., 20). The total amount of injected estradiol benzoate recovered in the urine of these animals as estrone ranged from 39 to 60 percent during a 4-day period. The estimates for estradiol were consistently low and without marked variation in any of the urine samples studied. Estriol was not detected. These results suggest that the uterus does not influence the quantity or kind of estrogen metabolites of gilt urine.

Exogenous estrogen maintains corpora lutea in the hypophysectomized rabbit (108), intact lamb (39) and sow (49, 86) and has an apparent luteolytic action in the cow (51, 53, 72, 132). In the hypophysectomized rat, exogenous estrogen causes an increase in the number of ovarian follicles and the amount of granulosa (101, 102, 130). Corpora lutea form in these rats following injections of PMS or HCG. According to Denamur and Mauléon (39), estradiol given from the day of induced ovulation in lambs maintained morphologically and histologically normal corpora lutea for about 50 days. Estrogen treatment did not maintain corpora lutea in lambs hypophysectomized on the day of induced ovulation; luteal regression was complete within 20 days. Similar results have been obtained in the gilt by du Mesnil du Buisson (86). Estradiol benzoate, at doses sufficient to maintain corpora lutea for prolonged periods in the intact gilt, failed to maintain corpora lutea following hypophysectomy. Daily injections of the estrogen were initiated either 2 days before or 6 days after hypophysectomy in the early luteal phase of the cycle (days 6 or 8) and total luteal regression occurred within 22 days after the previous estrus. Absence of luteal maintenance by estrogen in hypophysectomized lambs and gilts may indicate an indirect action of this hormone on the life span of the corpus luteum.

Results of recent experiments on the effect of injecting various pituitary hormones on luteal function generally have been negative in sheep and swine. Homogenates of fresh, whole pituitary glands from gilts at different stages of the estrous cycle and gestation did not alter the life span of corpora lutea in vivo or affect progesterone synthesis by swine luteal tissue in vitro (Anderson et al., 9; Duncan et al., 43). Short et al. (122) found that progesterone secretion of the corpus lutem was unaffected on the 9th and 15th day of the estrous cycle by injections of ovine prolactin, ovine LH, or chorionic gonadotropin. Furthermore, ovine FSH, growth hormone (GH), ACTH, thyrotropic hormone (TSH), PMS, fresh and acetone-dried ovine pituitaries or endometrical extracts failed to alter the secretory activity of the ovine corpus luteum, over a short period of time at the 9th day of the estrous cycle (Short, 121; Short et al., 122). Denamur and Mauléon (39) reported that bovine prolactin injected daily from the day of induced ovulation for 20 days did not maintain corpora lutea in intact lambs (up to 2,400 I.U. prolactin per day) or in lambs hypophysectomized on the day of ovulation (1,200 I.U. prolactin per day). In hypophysectomized rabbits (Kilpatrick et al., 68, 69; Rennie et al., 107) and in guinea pigs (Rowlands, 115) ovine prolactin has failed to maintain corpora lutea. However, ovine LH was luteotropic in hypophysectomized rabbits (Kilpatrick et al., 68, 69).

PMS, HCG or LH in the incubation medium stimulates in vitro synthesis of progesterone of corpora lutea from the ewe (Legault-Démare et al., 70); cow (Mason et al., 81; Savard and Casey, 119; Armstrong et al., 12; Armstrong, 11; Marsh and Savard, 76, 77); rat (Armstrong et al., 13), but little stimulating effect on progesterone synthesis from swine luteal tissue (Duncan et al., 43; Neill et al., 100). Increase in synthesis of progesterone by bovine luteal slices was effected by TPN and glucose-6-phosphate; DPN and DPNH did not alter this process. LH increased the incorporation of C^{14} acetate into progesterone 3 to 5 times, whereas addition of NADP and glucose-6-phosphate caused an 8-to 15-fold increase in progesterone production with no increase in the incorporation of C^{14} into the steroid (Marsh et al., 75; Mason et al., 81; Savard and Casey, 118). The minimum effective concentration of LH required for increased synthesis of progesterone in vitro was 0.01 - 0.02 mcg./gm. of bovine luteal tissue (Mason and Savard, 80). Prolactin did not produce a stimulatory effect on in vitro progesterone synthesis (Mason et al., 81) and the stimulating effect of FSH was attributed to small amounts of LH in the preparation (Mason and Savard, 81).

Microgram amounts of ovine LH in the incubation medium increased in vitro phosphorylase activity of luteal slices from pregnant cows (Marsh and Savard, 76). Luteinizing hormone not only maintained the high initial level of phosphorylase activity but was capable of increasing this activity after it had reached relatively low levels. Prolactin, ACTH, peroxide-in-activated LH and adenosine 3', 5' -monophosphate (3', 5' -AMP) were ineffective in stimulating phosphorylase activity, whereas ovine FSH and ovine GH were stimulatory. The stimulating effects of the latter hormones were explained as due to LH contamination. A corresponding increase in in vitro progesterone synthesis and phosphorylase activity occurred in bovine luteal slices when measured in the same luteal tissue. Marsh and Savard (77) reported that the addition of 3', 5' -AMP to incubating slices of bovine corpus luteum increased the rate of progesterone synthesis without increasing the phosphorylase activity of the tissue. The 2- to 3-fold stimulation by 3', 5' -AMP of the amount (mcg.) of progesterone synthesized was accompanied by a similar increase in the incorporation of acetate-1-C^{14} into the steroid. Results of incubating active and involuting bovine corpora lutea surgically removed at different stages of the estrous cycle were reported by Armstrong et al. (12). Maximal progesterone synthesis occurred from cows 4 to 13 days post estrus, declined gradually until day 18 and decreased to undetectable levels at day 19. Addition of LH to the incubation medium increased progesterone synthesis through day 18, but was ineffective on corpora lutea obtained from day 19 or later. Progesterone synthesis in corpora lutea obtained from day 19 or later was partially restored by addition of pregnenolone or of TPN plus glucose-6-phosphate to the incubation medium. TPN plus glucose-6-phosphate were only minimally effective in stimulating progesterone synthesis in corpora lutea from early stages of the estrous cycle (Armstrong, 11). It as suggested that possibly TPNH is not rate limiting in fresh luteal tissue but is rate limiting if tissue is deprived of oxygen or nutrient supply. Inactive corpora lutea were not deficient in stores of cholesterol but may be deficient in the enzyme system necessary for conversion of this probable precursor in progesterone synthesis. Simmons and Hansel (124) investigated luteal progesterone from heifers given different hormone treatments and suggested the occurrence of a specific bovine luteotropin hormone which is not bovine growth hormone, equine LH or ovine prolactin.

Armstrong and Greep (14) observed a stimulatory effect of LH upon the uptake of glucose by slices of luteinized rat ovaries and two-thirds of the utilized glucose was converted to lactic acid (Armstrong, 10). Lactic acid formation was stimulated by in vivo LH and in proportion to its stimulation of glucose uptake. In these experiments FSH and prolactin did not stimulate glucose uptake except at levels which could be caused by LH contamination. When the LH was given intravenously to rats 4 hr. prior to autopsy, there was a significant increase in the conversion of glucose to CO_2 and lipid, as well as an overall increase in the uptake of glucose by the luteal slices. However, when LH was added directly to the incubation medium there resulted an increased progesterone synthesis without affecting the rate of glucose metabolism (Armstrong et al., 13). Increased glycolysis appeared to be an effect rather than a cause for the increased synthesis of luteal progesterone. Armstrong (11) found that stimulation of glycolysis following LH was involved in the replenishment of stores of lipid precursors which have been depleted as a result of the stimulatory action of LH upon progesterone synthesis. It was proposed that LH possibly mobilizes lipid stores for progesterone synthesis by the corpora lutea and these lipid stores are replenished both from cholesterol and long chain free fatty acids brought to the tissue by the plasma.

In the ewe, corpora lutea, induced by ovine pituitary extract at different luteal phases of the estrous cycle, regress at the same time as the natural corpora lutea, even after the natural corpora lutea have been removed (Inskeep et al., 65). It was concluded that the life span of corpora lutea in this species is determined by a factor extrinsic to the gland itself. However, in the gilt, induced and natural corpora lutea of different ages on the same ovary retain their luteal life spans of approximately one estrous cycle (Neill and Day, 99). Thus, an intrinsic mechanism determining the life of the corpus luteum possibly at the time of luteal formation is likely in this species. Both natural and induced corpora lutea persist in hysterectomized gilts.

There appear to be species differences in the relationship between the level of ovarian and subsequent luteal function and the degree of pituitary activity (Nalbandov, 97). For example, the events of ovulation, whether induced or spontaneous, are sufficient to cause the formation and maintenance of corpora lutea in the pig for a period of time characteristic of this species. In the ewe, however, the physiologic events leading to the development of these glands do not appear adequate for their maintenance without supplemental pituitary stimulation. It is possible, in species producing functional corpora lutea, that luteolysis during the estrous cycle is initiated as a result of an inhibitory humoral or neurohumoral stimulus of uterine origin acting on the anterior pituitary by way of the CNS. This action could lead either to inhibition of hypophysial luteotropin or alteration in gonadotropic complex activity and would thereby induce luteal regression. During pregnancy and following hysterectomy, this uterine inhibition is absent in some species and as a result functional corpora lutea persist (Nalbandov, personal communication).

Increasing attention has been given to the role of the uterus in alteration of pituitary and gonad function. Maintenance of the functional corpus luteum during at least the initial phases of gestation is well recognized in several species (Amoroso and Finn, 2). Hysterectomy alters ovarian function which results in persistence of the corpus luteum in the guinea pig (Butcher et al., 27; Loeb, 71; Rowlands, 114); ewe (Denamur and Mauléon, 39; Moor and Rowson, 91; Wiltbank and Casida, 131); sow (Anderson et al., 6; Anderson et al., 7; du Mesnil du Buisson and Dauzier, 87; Neill and Day, 99; Spies et al., 127) and cow (Anderson and Bowerman, 4; Anderson et al., 3; Armstrong and Hansel, 15; Wiltbank and Casida, 131; Wiltbank et al., 133). The corpora lutea following hysterectomy are maintained for a period approaching or exceeding the length of gestation in these species. However, that same operation has no apparent effect on ovarian function in the ferret, opossum, monkey, woman or unmated rat, mouse and rabbit (Amoroso and Finn, 2; Anderson et al., 5). Hysterectomy performed at different phases of the estrous cycle has been reported recently in the gilt (Anderson et al., 7); guinea pig (Rowlands, 114); ewe (Moor and Rowson, 91) and at different stages of pseudopregnancy in the rat (Melampy et al., 82). Silbiger and Rothchild (123) suggest that hysterectomy in the rat results in decreased secretion of both FSH and LH and that a decrease in the luteolytic effectiveness of the pituitary is associated with the diminished secretion of LH.

In the gilt, removal of the uterus at estrus or days 5, 10, 14 and in a majority at day 16, resulted in formation and maintenance of corpora lutea. Estrus and ovulation occurred within a few days when the uterus was removed at day 18. Although morphologic changes associated with luteal regression occurred in hysterectomized gilts during the interval between days 16 and 18, the presence of a luteolytic or absence of luteotropic action may be initiated at an earlier stage of the cycle. Hysterectomy as late as day 15 of the cycle prevented impending estrus and ovulation in guinea pigs (Rowlands, 114) and, in ewes (Moor and Rowson, 91), arrested the involutionary changes in luteal cells, provided the corpus luteum was still functional at the time of the operation. In rats, hysterectomy on days 5, 9 and 11 of pseudopregnancy resulted in prolongation of the life span of corpora lutea to approximately that of normal gestation, whereas luteal regression was initiated somewhat earlier when the uterus was removed at day 13 (Melampy et al., 82). Enucleation of the persisting corpora lutea in hysterectomized gilts (Anderson et al., 9) and heifers (Anderson and Bowerman, 4) is followed within a few days by estrus, ovulation and maintenance of the newly formed corpora lutea.

Secretion of a hypophysial luteotropin for maintenance of corpora lutea for prolonged periods in the hysterectomized ewe and gilt is evident by luteal regression following hypophysectomy in these animals. Denamur and Mauléon (39) found that corpora lutea were in the process of regression or completely regressed 20 days after hysterectomizing and hypophysectomizing ewes on the day of ovulation. Furthermore, luteal regression was complete within 20 days in ewes hypophysectomized 20 days after hysterectomy. Du Mesnil du Buisson and Léglise (88) reported that luteal regression began within 5 days and was complete by 10 to 11 days in gilts previously hysterectomized during the early luteal phase (days 4 to 8) and hypophysectomized 22 days after the beginning of estrus. Similar results were obtained when gilts were hypophysectomized 29, 46, 97 and 99 days after hysterectomy (du Mesnil du Buisson, 86). Complete luteal regression also occurred within 20 days when hypophysectomies were performed at estrus and followed by hysterectomies in the early luteal phase of the cycle (du Mesnil du Buisson, 86). Maintenance of corpora lutea in the hysterectomized ewe and gilt is not possible in the absence of the pituitary. Persistence of corpora lutea following hypophysectomy in previously hysterectomized guinea pigs indicates that removal of the uterus does not affect luteal function by secretion of a pituitary luteotropin this species (Rowlands, 115). In experiments of Deanesly and Perry (37), both progesterone and reserpine caused regression of corpora lutea in hysterectomized guinea pigs. They concluded that in the guinea pig the corpora lutea of hysterectomy do not function independently of the pituitary.

Short (121) has proposed a system of dual control of the life span of the corpus luteum for the ewe by a pituitary luteotropin and a uterine luteolysin. In this scheme, a pituitary "on" mechanism would stimulate and maintain the corpus luteum life span but perhaps not its secretory activity by a single release of a pituitary luteotropin at the time of ovulation. However, the luteotropin may be released into the circulation continuously. A uterine "off" mechanism would determine the length of the estrous cycle by providing a luteolysin. Of the two mechanisms, the uterine luteolysin would have the overriding effect.

Subtotal hysterectomy in the guinea pig (Butcher et al., 27); gilt (Anderson et al., 6); heifer (Anderson et al., 8) and ewe (Moor and Rowson, 91) results in luteal regression and continuation of estrous cycles. However, the duration of the estrous cycle in these species is affected by the proportion of uterus removed. Recent investigations in the ewe by Moor and Rowson (91) showed that one-half of the cycles were extended beyond 20 days when one uterine horn was removed, whereas one-fourth of the cycles were extended when only the distal half of one horn was removed. Uterine and endometrial autotransplants in the guinea pig (Butcher et al., 26) and uterine autotransplants in the pig (Anderson et al., 7; du Mesnil du Buisson and Rombauts, 89) results in continuation of estrous cycles and, in rats, (Melampy et al., 82) reduces the duration of pseudopregnancy. A functional endometrium, as evaluated histologically, appears to be necessary for luteal regression in these species.

Unilateral regression of corpora lutea has been observed on the side of the uterine fragment in partially hysterectomized gilts (du Mesnil du Buisson, 85). In gilts mated after one uterine horn was severed from the uterine body, early failure of pregnancy occurred in the intact horn (du Mesnil du Buisson, 84). Gestation continued in these animals if the non-gravid horn was removed by day 14, but pregnancy was terminated when unilateral hysterectomy was postponed until after day 16 (du Mesnil du Buisson, 84). Unilateral luteal regression occurred on the side of the non-gravid horn in gilts that became pregnant with either a whole or anterior half or quarter of a sterile horn present (Rathmacher and Anderson, 106). Normal pregnancy and maintenance of corpora lutea on both ovaries occurred in unilaterally hysterectomized animals. It is apparent that the non-gravid horn is responsible for the termination of pregnancy through a luteolytic action which it initiates. The physiologic basis of this action is unknown, but it is possible that ovarian-uterine vascular relationships may be involved in the initiation of this luteolysis.

The duration of anestrum was prolonged more than 100 days in 6 of 8 intact gilts injected with an optimum total of 35 mg. of estradiol benzoate over a 7-day period beginning on days 7, 9 or 12 of the cycle (du Mesnil du Buisson, 86). Estrogen treatment beginning at day 14 had no effect on the length of the estrous cycle. Corpora lutea were maintained in both ovaries in estrogen-treated unilaterally pregnant gilts with one empty uterine horn at 30, 55 or 90 to 103 days of gestation. Therefore, estradiol benzoate suppresses luteolytic action, not only during the estrous cycle, but also in unilaterally pregnant gilts in which the non-gravid uterine horn would otherwise effect a unilateral regression of corpora lutea on the side of the non-gravid horn. Du Mesnil du Buisson and Rombauts (90) found that gestation continued in a majority of gilts in which all of the uterus was removed except one embryo and its corresponding portion of uterine horn on the 12th day of pregnancy. When a certain number of embryos were removed with or without the corresponding portion of the uterine horns around the 40th or 80th day of gestation, pregnancy continued in most animals in which some embryos with corresponding uterine horns were removed, whereas very few gilts remained pregnant when only some embryos were removed. The portion of empty uterine horn apparently had a negative influence on maintenance of pregnancy after the first 40 days, but uterine luteolytic activity of the empty horn was not evident on the corresponding ovary at this time.

Surgical removal of embryos during the first 12 days in the ewe resulted in subsequent normal estrous behavior, whereas removal of embryos on the 13th and 14th days after estrus resulted in a marked extension of the luteal life span (Moor and Rowson, 91). Furthermore, transfer of 12 or 13 day embryos to the uterus of non-pregnant recipient ewes on the 12th day of their cycle resulted in normal pregnancies. Embryonic loss frequently occurred when 13 day embryos were transferred to day 13 recipient ewes. It was suggested that the life span of the corpus luteum in this species was not irrevocably determined until after the 12th day of the estrous cycle.

Estrous cycles are altered by uterine distention in the rat (Selye, 120); guinea pig (Donovan and Traczyk, 42; Moore, 92); cow (Armstrong and Hansel, 15; Chatterjee and Luktuke, 29; Hansel, 54; Hansel and Wagner, 55; Yamauchi and Nakahara, 134) and ewe (Inskeep et al., 64; Moore and Nalbandov, 93; Nalbandov et al., 98) but not in the pig (Anderson, 3). Uterine innervation in these species can play a role in changing ovarian function, possibly by affecting central nervous system activity and thereby pituitary hormone secretion or possibly by a more direct action on the ovary. However, mechanisms by which uterine distention modifies the cycle may be unrelated to those mechanisms affecting the physiological processes in the formation, maintenance and regression of the corpus luteum during the normal estrous cycle or during pregnancy.

Summary

Follicular growth and maturation are dependent upon pituitary gonadotropins; ovulation is followed by further proliferation of granulosa and theca cells which form the corpus luteum. In the ewe and sow the ovary is capable of ovulation when the pituitary is removed at or just prior to ovulation, the corpus luteum which develops subsequently is maintained for the approximate duration of the estrous cycle. It has been proposed that perhaps a pituitary luteotropin, if required, is secreted for a brief period at the time of ovulation and is sufficient for the development of the corpus luteum of that cycle. Luteinizing hormone is required for the processes of follicular maturation and ovulation as indicated by experimental induction of ovulation and by depletion of pituitary gonadotropin content prior to ovulation. The necessity for a luteotropin, whether this is luteinizing hormone or an unidentified pituitary luteotropin, is not clearly established for the initial development of the corpus luteum during the estrous cycle. Maintenance of the corpus luteum in the pregnant or hysterectomized animal is dependent upon an apparent continuous requirement of pituitary gonadotropin with luteotropic action.

The role of the uterus in altering the life of the corpus luteum in the cow, sow and ewe is evident by extension of the luteal life span during pregnancy and following hysterectomy, and, in the case of the sow, luteolytic action of a non-gravid portion of the uterus in partially hysterectomized or unilaterally pregnant animals. The physiologic basis of unilateral regression of corpora lutea is unknown. It is possible that ovarian-uterine vascular relationships may be involved in the initiation of this luteolysis. In subtotally hysterectomized animals, estrous cycles occur, however, the duration of the cycle is altered by the proportion of uterus removed. Estrous cycles occur following autotransplantation of the uterus in the guinea pig and sow and in rats the duration of pseudopregnancy is reduced. A functional endometrium appears necessary for luteal regression in these species. It may be that in the cycling female the uterus maintains a positive inhibition of pituitary luteotropin secretion and during pregnancy or following hysterectomy, this inhibition is lacking; hence, the persistence of corpora lutea.

Literature Cited

(1) Aldred, J. P., Sammelwitz, P. H. and Nalbandov, A. V.
1961. Mechanism of Formation of Corpora Lutea in Guinea-Pigs. Jour. Reprod. Fertil. 2: 394.

(2) Amoroso, E. C. and Finn, C. A.
1962. The Ovary. Academic Press, London. p. 451.

(3) Anderson, L. L.
1962. Effect of Uterine Distention on the Estrous Cycle of the Gilt. Jour. Anim. Sci. 21: 597.

(4) Anderson, L. L. and Bowerman, A. M.
1963. Utero-Ovarian Function in Oxytocin-Treated Heifers. Jour. Anim. Sci. 22: 1136. (Abstr.).

(5) Anderson, L. L., Bowerman, A. M., and Melampy, R. M.
1963a. Advances in Neuroendocrinology. Univ. Ill. Press, Urbana. p. 345.

(6) Anderson, L. L., Butcher, R. L., and Melampy, R. M.
1961. Subtotal Hysterectomy and Ovarian Function in Gilts. Endocrinology. 69: 571.

(7) Anderson, L. L., Butcher, R. L., and Melampy, R. M.
1963b. Uterus and Occurrence of Oestrus in Pigs. Nature 198: 311.

(8) Anderson, L. L., Neal, F. C., and Melampy, R. M.
1962. Hysterectomy and Ovarian Function in Beef Heifers. Amer. Jour. Vet. Res. 23: 794.

(9) Anderson, L. L., Schultz, J. R., and Melampy, R. M.
1964. Gonadotropins. Their Chemical and Biological Properties and Secretory Control. Freeman and Co., San Francisco. p. 171.

(10) Armstrong, D. T.
1963. Stimulation of Glycolytic Activity of Rat Corpus Luteum Tissue by Luteinizing Hormone. Endocrinology. 72: 908.

(11) Armstrong, D. T.
1964. Corpus Luteum Metabolism and Steroid Biogenesis. Presented at the Second International Congress of Endocrinology, London, 1964.

(12) Armstrong, D. T., Black, D. L., and Cone, C. E.
1964a. Metabolic Studies with Active and Involuting Bovine Corpora Lutea. Fed. Proc. 23: 462. (Abstr.).

(13) Armstrong, D. T., O'Brien, J., and Greep, R. O.
1964b. Effects of Luteinizing Hormone on Progestin Biosynthesis in the Luteinized Rat Ovary. Endocrinology 75: 488.

(14) Armstrong, D. T., and Greep, R. O.
1962. Effect of Gonadotrophic Hormones on Glucose Metabolism by Luteinized Rat Ovaries. Endocrinology 70: 701.

(15) Armstrong, D. T., and Hansel, W.
1959. Alteration of the Bovine Estrous Cycle with Oxytocin. Jour. Dairy Sci. 42: 533.

(16) Asdell, S. A.
1955. Cattle Fertility and Sterility (1st ed.). Little, Brown and Co., Boston.

(17) Asdell, S. A., de Alba, J., and Roberts, S.J.
1949. Studies on the Estrous Cycle of Dairy Cattle: Cycle Length, Size of Corpus Luteum and Endometrial Changes. Cornell Vet. 39: 389.

(18) Astwood, E. B.
1941. The Regulation of Corpus Luteum Function by Hypophysial Luteotrophin. Endocrinology 28:309.

(19) Barker, W. L.
1951. A Cytochemical Study of Lipids in Sows' Ovaries During the Estrous Cycle. Endocrinology 48: 772.

(20) Bowerman, A. M., Anderson, L. L., and Melampy, R. M.
1964. Urinary Estrogens in Cycling, Pregnant, Ovariectomized and Hysterectomized Gilts. Iowa State Jour. Sci. 38: 437.

(21) Boyd, W. L.
1925. A Study of the Physiologic and Pathologic Changes Occurring in the Reproductive Organs of the Cow Following Parturition. Minn. Agr. Expt. Sta. Tech. Bul. 23.

(22) Bradbury, J. T., Brown, W. E., and Gray, L. A.
1950. Maintenance of the Corpus Luteum and Physiologic Actions of Progesterone. Recent Progr. Hormone Res. 5: 151.

(23) Brambell, F. W. R.
1956. Ovarian Changes. Marshall's Physiology of Reproduction (3rd ed.). Vol. 1, pt. 1. Longmans, Green and Co., London.

(24) Brinkley, H. J., Norton, H. W., and Nalbandov, A. V.
1964a. Is Ovulation Alone Sufficient to Cause Formation of Corpora Lutea? Endocrinology 74: 14.

(25) Brinkley, H. J., Norton, H. W., and Nalbandov, A. V.
1964b. Role of a Hypophysial Luteotrophic Substance in the Function of Porcine Corpora Lutea. Endocrinology 74: 9.

(26) Butcher, R. L., Chu, K. Y., and Melampy, R. M.
1962a. Effect of Uterine Autotransplants on the Estrous Cycle in the Guinea Pig. Endocrinology 70: 442.

(27) Butcher, R. L., Chu, K. Y., and Melampy, R. M.
1962b. Utero-Ovarian Relationships in the Guinea Pig. Endocrinology 71: 810.

(28) Casida, L. E., and McKenzie, F. F.
1932. The Oestrous Cycle of the Ewe: Histology of the Genital Tract. Mo. Agr. Expt. Sta. Res. Bul. 170.

(29) Chatterjee, S. N., and Luktuke, S. N.
1961. Studies on Induction of Sterility in Scrub Cows by Intrauterine

Introduction of Spring Pessaries. Jour. Reprod. Fertil. 2: 196. (Abstr.).

(30) Chester-Jones, I., Ball, and J. N.
1962. The Ovary. Academic Press, London. p. 361.

(31) Corner, G. W.
1915. The Corpus Luteum of Pregnancy As It Is in Swine. Carnegie Inst. Contr. Embryol. 5: 69.

(32) Corner, G. W.
1919. On the Origin of the Corpus Luteum of the Sow from Both Granulosa and Theca Interna. Amer. Jour. Anat. 26: 117.

(33) Corner, G. W.
1920. On the Widespread Occurrences of Reticular Fibrils Produced by Capillary Endothelium. Carnegie Inst. Contr. Embryol. 9: 85.

(34) Corner, G. W.
1921. Cyclic Changes in the Ovaries and Uterus of the Sow and Their Relation to the Mechanism of Implantation. Carnegie Inst. Contr. Embryol. 13: 117.

(35) Corner, G. W.
1948. Alkaline Phosphatase in the Ovarian Follicle and in the Corpus Luteum. Carnegie Inst. Contr. Embryol. 32: 1.

(36) Cowie, A. T., Daniel, P. M., Prichard, M. M. L., and Tindal, J. S.
1963. Hypophysectomy in Pregnant Goats, and Section of the Pituitary Stalk in Pregnant Goats and Sheep. Endocrinology 28: 93.

(37) Deanesly R., and Perry, J. S.
1964. Corpus Luteum Control in Hysterectomized Guinea-Pigs. Endocrinology.

(38) Denamur, R., and Martinet, J.
1961. Effets de l'hypophysectomie et de la section de la tige pituitaire sur la gestation de la brebis. Ann. Endocrinology (Paris) 22: 755.

(39) Denamur, R., and Mauléon, P.
1963a. Contrôle endocrinien de la persistence du corps jaune chez les ovins. Compt. Rend. Acad. Sci. 257: 527.

(40) Denamur, R., and Mauléon, P.
1963b. Effets de l'hypophysectomie sur la morphologie et l'histologie du corps jaune des ovins. Compt. Rend. Acad. Sci. 257: 264.

(41) Donovan, B. T.
1963. The Effect of Pituitary Stalk Section on Luteal Function in the Ferret. Endocrinology 27: 201.

(42) Donovan, B. T., and Traczyk, W.
1960. Uterine Distention and the Vaginal Cycle in the Guinea Pig. Jour. Physiol. 154: 50. (Abstr.).

(43) Duncan, G. W., Bowerman, A. M., Anderson, L. L., Hearn, W. R., and Melampy, R. M.
1961. Factors Influencing in vitro Synthesis of Progesterone. Endocrinology 68: 199.

(44) Evans, H. M., Simpson, M. E., and Lyons, W. R.
1941a. Influence of Lactogenic Preparations on Production of Traumatic Placentoma in the Rat. Proc. Soc. Expt. Biol. Med. 46: 586.

(45) Evans, H. M., Simpson, M. E., Lyons, W. R., and Turpeinen, K.
1941b. Anterior Pituitary Hormones Which Favor the Production of Traumatic Uterine Placentomata. Endocrinology 28: 933.

(46) Everett, J. W.
1956. Functional Corpora Lutea Maintained for Months by Autografts of Rat Hypophyses. Endocrinology 58: 786.

(47) Foley, R. C., and Greenstein, J. S.
1958. Cytological Changes in the Bovine Corpus Luteum During Early Pregnancy. Reproduction and Infertility. III Symposium. Pergamon Press, London. p. 88.

(48) Foley, R. C., and Reece, R. P.
1953. Histological Studies of the Bovine Uterus, Placenta and Corpus Luteum, Univ. Mass. Agr. Expt. Sta. Bul. 468.

(49) Gardner, M. L., First, N. L., and Casida, L. E.
1963. Effect of Exogenous Estrogens on Corpus Luteum Maintenance in Gilts. Jour. Anim. Sci. 22: 132.

(50) Grant, R.
1934. Studies on the Physiology of Reproduction in the Ewe. III. Gross Changes in the Ovaries. Trans. Roy. Soc. Edinb. 58: 36.

(51) Greenstein, J. S., Murray, R. W., and Foley, R. C.
1958. Effect of Exogenous Hormones on the Reproductive Processes of the Cycling Dairy Heifer. Jour. Dairy Sci. 41: 1834.

(52) Hammond, J.
1927. The Physiology of Reproduction in the Cow. The University Press, Cambridge.

(53) Hammond, J., Jr., and Day, F. T. 1944. Oestrogen Treatment of Cattle: Induced Lactation and Other Effects. Endocrinology 4: 53.

(54) Hansel, W. 1959. Further Studies on the Regulation of the Bovine Estrous Cycle by Oxytocin Injections. Jour. Dairy Sci. 42: 940. (Abstr.).

(55) Hansel, W., and Wagner, W. C. 1960. Luteal Inhibition in the Bovine as a Result of Oxytocin Injections, Uterine Dilatation, and Intrauterine Infusions of Seminal and Preputial Fluids. Jour. Dairy Sci. 43: 796.

(56) Harrison, R. J. 1946. The Early Development of the Corpus Luteum in the Mare. Jour. Anat. 80: 160.

(57) Harrison, R. J. 1948a. The Changes Occurring in the Ovary of the Goat During the Estrous Cycle and in Early Pregnancy. Jour. Anat. 82: 21.

(58) Harrison, R. J. 1948b. The Development and Fate of the Corpus Luteum in the Vertebrate Series. Biol. Rev. 23: 296.

(59) Harrison, R. J. 1962. The Ovary. Academic Press, London. p. 143.

(60) Hett, J. 1933. Handbuch der verbleichenden Anatomie der Wirbeltiere. Urban and Schwarzenberg, Berlin. p. 253.

(61) Hilliard, J., Endröczi, E., and Sawyer, C. H. 1961. Stimulation of Progestin Release From Rabbit Ovary *in vivo*. Proc. Soc. Expt. Biol. Med. 108: 154.

(62) Höfliger, N. 1948. Das Ovar des Rindes in den verschiedenen Lebensperioden unter besonderer Berücksichtigung seiner funktionellen Feinstruktur. Acta Anat. 3, Supp. 5: 1.

(63) Holmstrom, E. G., and Jones, W. J. 1949. The Experimental Production of Menorrhagia by Administration of Gonadotropins. Amer. Jour. Obst. and Gyn. 58: 308.

(64) Inskeep, E. K., Oloufa, M. M., Howland, B. E., Pope, A. L., and Casida, L. E. 1962. Effect of Experimental Uterine Distention on Estrual Cycle Lengths in Ewes. Jour. Anim. Sci. 21: 331.

(65) Inskeep, E. K., Oloufa, M. M., Pope, A. L., and Casida, L. E. 1963. Functional Capabilities of Experimentally Induced Corpora Lutea in Ewes. Jour. Anim. Sci. 22: 159.

(66) Jongh, S. E. de, and Wolthuis, O. L. 1964. Factors Determining Cessation of Corpus Luteum Function; the Possible Role of Oestradiol and Progesterone. Acta Endocrinology Supp. 90: 125.

(67) Kidder, H. E., Casida, L. E., and Grummer, R. H. 1955. Some Effects of Estrogen Injections on the Estrual Cycle of Gilts. Jour. Anim. Sci. 14: 470.

(68) Kilpatrick, R., Armstrong, D. T., and Greep, R. O. 1962. Maintenance of the Corpus Luteum. Lancet II: 462.

(69) Kilpatrick, R., Armstrong, D. T., and Greep, R. O. 1964. Maintenance of the Corpus Luteum by Gonadotrophins in the Hypophysectomized Rabbit. Endocrinology 74: 453.

(70) Legault-Démare, J., Mauléon, P., and Suarez-Soto, M. 1960. Etude de L'activite biologique in vitro des hormones gonadotropes. Acta Endocr. 34: 163.

(71) Loeb, L. 1923. The Effect of Extirpation of the Uterus on the Life and Function of the Corpus Luteum in the Guinea Pig. Proc. Soc. Expt. Biol. Med. 20: 441.

(72) Loy, R. G., Zimbelman, R. G., and Casida, L. E. 1960. Effects of Injected Ovarian Hormones on the Corpus Luteum of the Estrual Cycle in Cattle. Jour. Anim. Sci. 19: 175.

(73) McNutt, G. W. 1924. The Corpus Luteum of the Ox Ovary in Relation to the Estrous Cycle. Jour. Amer. Vet. Med. Assoc. 65: 556.

(74) McNutt, G. W. 1927. The Corpus Luteum of Pregnancy in the Domestic Cow (*Bos taurus*) and a Brief Discussion of Cyclical Ovarian Changes. Jour. Amer. Vet. Med. Assoc. 72: 286.

(75) Marsh, J. M., Mason, N. R., and Savard, K. 1961. An *in vitro* Action of Gonadotropin. Fed. Proc. 20: 187.

(76) Marsh, J. M., and Savard, K. 1964a. The Activation of Luteal Phosphorylase by Luteinizing Hormone. Jour. Biol. Chem. 239: 1.

(77) Marsh, J. M., and Savard, K.
1964b. The Effect of 3', 5' -AMP on Progesterone Synthesis. Fed. Proc. 23: 462.

(78) Marshall, F. H. A.
1903. The Oestrous Cycle and the Formation of the Corpus Luteum in the Sheep. Phil. Trans. Roy. Soc. London, Ser. B. 196: 47.

(79) Marshall, F. H. A.
1910. The Physiology of Reproduction (1st ed.). Longmans, Green and Co., London.

(80) Mason, N. R., and Savard, K.
1964. Specificity of Gonadotropin Stimulation of Progesterone Synthesis in Bovine Corpus Luteum in vitro. Endocrinology 74: 664.

(81) Mason, N. R., Marsh, J. M., and Savard, K.
1962. An Action of Gonadotropins in vitro. Jour. Biol. Chem. 237: 1801.

(82) Melampy, R. M., Anderson, L. L., and Kragt, C. L.
1964. Uterus and Life Span of Rat Corpora Lutea. Endocrinology 74: 501.

(83) Melampy, R. M., and Gay, D. G.
1958. A Study of the Weight of the Corpus Luteum During Pregnancy in the Cow. Iowa State College Jour. Sci. 33: 37.

(84) du Mesnil du Buisson, F.
1961a. Possibilité d'un fonctionnement dissemblable des ovaries pendant la gestation chez la Truie. Compt. Rend. Acad. Sci. 253: 727.

(85) du Mesnil du Buisson, F.
1961b. Regression unilatérale des corps jaunes après hysterectomie partielle chez la Truie. Ann. Biol. Anim. Bioch. Biophys. 1: 105.

(86) du Mesnil du Buisson, F.
1964. Thesis. (in press).

(87) du Mesnil du Buisson, F., and Dauzier, L.
1959. Controle mutuel de l'uterus et de l'ovaire chez la Truie. Ann. Zootech. Supp. p. 147.

(88) du Mesnil du Buisson, F., and Léglise, P. C.
1963. Effet de l'hypophysectomie sur les corps jaunes de la Truie. Résultats préliminaries. Compt. Rend. Acad. Sci. 257: 261.

(89) du Mesnil du Buisson, F., and Rombauts. P.
1963a. Effet d'autotransplants uterins sur le cycle oestrien de la Truie. Compt. Rend. Acad. Sci. 256: 4984.

(90) du Mesnil du Buisson, F., and Rombauts, P.
1963b. Réduction expérimentale du nombre des foetus au ccurs de la gestation de la Truie et maintien des corps jaunes. Ann. Biol. Anim. Bioch. Biophys. 3: 445.

(91) Moor, R. M., and Rowson, L. E. A.
1964. Influence of the Embryo and Uterus on Luteal Function in the Sheep. Nature. 201: 522.

(92) Moore, W. W.
1961. Effect of Uterine Distention on the Estrous Cycle of the Guinea Pig. Physiologist. 4: 76. (Abstr.).

(93) Moore, W. W., and Nalbandov, A. V.
1953. Neurogenic Effects of Uterine Distention on the Estrous Cycle of the Ewe. Endocrinology 53: 1.

(94) Moore, W. W., and Nalbandov, A. V.
1955. Maintenance of Corpora Lutea in Sheep with Lactogenic Hormone. Endocrinology 13: 18.

(95) Moss, S., Wrenn, T. R., and Sykes. J. F.
1954a. Alkaline Phosphatase, Glycogen and Periodic Acid-Schiff Positive Substances in the Bovine Uterus During the Estrous Cycle. Endocrinology 55: 261.

(96) Moss, S., Wrenn, T. R., and Sykes, J. F.
1954b. Some Histological and Histochemical Observations of the Bovine Ovary During the Estrous Cycle. Anat. Rec. 120: 409.

(97) Nalbandov, A. V.
1961. Comparative Physiology and Endocrinology of Domestic Animals. Recent Progr. Hormone Res. 17: 119.

(98) Nalbandov, A. V., Moore, W. W., and Norton, H. W.
1955. Further Studies on the Neurogenic Control of the Estrous Cycle by Uterine Distention. Endocrinology 56: 225.

(99) Neill, J. D., and Day, B. N.
1964. Relationship of Developmental Stage to Regression of the Corpus Luteum in Swine. Endocrinology 74: 355.

(100) Neill, J. D., Day, B. N., and Duncan, G.W.
1964. Gas Chromatographic Determination of Progestins in Tissue and Blood. Steroids 4: 699.

(101) Payne, R. W., and Hellbaum, A. A.
1955. The Effect of Estrogens on the Ovary of the Hypophysectomized Rat. Endocrinology 57: 193.

(102) Pencharz, R. I.
1940. Effect of Estrogens and Androgens Alone and in Combination with Chorionic

Gonadotropin on the Ovary of the Hypophysectomized Rat. Science 91: 554.

(103) Pratt, J. P.
1935. The Human Corpus Luteum. Arch. Path. Lab. Med. 19:380.

(104) Quilligan, E. J., and Rothchild, I.
1960. The Corpus Luteum-Pituitary Relationship: The Luteotrophic activity of Homotransplanted Pituitaries in Intact Rats. Endocrinology 67: 48.

(105) Quinlan, J., and Maré, G. S.
1931. The Physiological Changes in the Ovary of the Merino Sheep in South Africa and the Practical Application in Breeding. 17th Rpt. Vet. Res. S. Africa. 663.

(106) Rathmacher, R. P., and Anderson, L. L.
1963. Sterile Uterine Horn and Embryonic Survival in Pigs. Jour. Anim. Sci. 22: 1139. (Abstr.).

(107) Rennie, P., Davies, J., and Friedrich, E.
1964. Failure of Ovine Prolactin to Show Luteotrophic or Luteolytic Effects in the Rabbit. Endocrinology 75: 622.

(108) Robson, J. M.
1937. Maintenance by Oestrin of the Luteal Function in Hypophysectomized Rabbits. Jour. Physiol. 90: 435.

(109) Rombauts, P., and du Mesnil du Buisson, F.
1964. Excrétion d'oestrogénes après hystérectomie durant la gestation ou pendant le cycles oestral. Compt. Rend. Acad. Sci. 258: 5076.

(110) Rothchild, I.
1960a. The Corpus Luteum-Pituitary Relationship: The Association Between the Cause of Luteotrophin Secretion and the Cause of Follicular Quiescence During Lactation; the Basis for a Tentative Theory of the Corpus Luteum-Pituitary Relationship in the Rat. Endocrinology 67: 9.

(111) Rothchild, I.
1960b. The Corpus Luteum-Pituitary Relationship: The Lack of an Inhibiting Effect of Progresterone on the Secretion of Pituitary Luteotrophin. Endocrinology 67: 54.

(112) Rothchild, I.
1962. Positive Feedback in Luteal Phase Regulation of Luteotrophin Secretion. Proc. I.U.P.S. XXII Inter. Congr. I: 746.

(113) Rothchild, I.
1964. An Explanation for the Cause of Luteolysis in the Rat and its Possible Application to Other Species. Presented at the Second International Congress of Endocrinology, London. 1964.

(114) Rowlands, I. W.
1961. Effect of Hysterectomy at Different Stages in the Life Cycle of the Corpus Luteum in the Guinea-Pig. Jour. Reprod. Fertil. 2: 341.

(115) Rowlands, I. W.
1962. The Effect of Oestrogens, Prolactin and Hypophysectomy on the Corpora Lutea and Vagina of Hysterectomized Guinea-Pigs. Endocrinology 24: 105.

(116) Sammelwitz, P. H., Aldred, J. P., and Nalbandov, A. V.
1961. Mechanisms of Maintenance of Corpora Lutea in Pigs and Rats. Jour. Reprod. Fertil. 2: 387.

(117) Sammelwitz, P. H., and Nalbandov. A. V.
1958. Progesterone-Induced Regression of Corpora Lutea in Pregnant and Cycling Gilts. Jour. Anim. Sci. 17: 1233. (Abstr.).

(118) Savard, K., and Casey, P. J.
1963. Differing Actions of NADPH and Tropic Hormones (LH and ACTH) on Steroidogenesis. Fed. Proc. 22: 530.

(119) Savard, K., and Casey, P. J.
1964. Effects of Pituitary Hormones and NADPH on Acetate Utilization in Ovarian and Adrenocortical Tissue. Endocrinology 74: 599.

(120) Selye, H.
1933. Influence of the Uterus on Ovary and Mammary Gland. Proc. Soc. Exp. Biol. Med. 31: 488.

(121) Short, R. V.
1964. Ovarian Steroid Synthesis and Secretion _in vivo_. Recent Progr. Hormone Res. 20: 303.

(122) Short, R. V., McDonald, M. F., and Rowson, L. E. A.
1963. Steriods in the Ovarian Venous Blood of Ewes Before and After Gonadotrophic Stimulation. Endocrinology 26: 155.

(123) Silbiger, M., and Rothchild, I.
1963. The Influence of the Uterus on the Corpus Luteum-Pituitary Relationship in the Rat. Acta Endrocinology 43: 521.

(124) Simmons, K. R., and Hansel, W.
1964. Nature of the Luteotropic Hormone in the Bovine. Jour. Anim. Sci. 23: 136.

(125) Smith, V. R., McShan, W. H., and Casida, L. E.
1957. On Maintenance of the Corpora Lutea of the Bovine with Lactogen. Jour. Dairy Sci. 40: 443.

(126) Solomons, B., and Gatenby, J. W. B.
1924. Notes on the Formation, Structure and Physiology of the Corpus Luteum of Man, the Pig and the Duck-Billed Platypus. Jour. Obst. Gyn. British Emp. 31: 580.

(127) Spies, H. G., Zimmerman, D. R., Self, H. L., and Casida, L. E.
1960. Effect of Exogenous Progesterone on the Corpora Lutea of Hysterectomized Gilts. Jour. Anim. Sci. 19: 101.

(128) Warbritton, V.
1934. The Cytology of the Corpora Lutea of the Ewe. Jour. Morphol. 56: 181.

(129) Weeth, H. J., and Herman, H. A.
1952. Histological and Histochemical Study of the Bovine Oviducts, Uterus and Placenta. Mo. Agr. Expt. Sta. Res. Bul. 501.

(130) Williams, P. C.
1940. Effect of Stilboestrol on the Ovaries of Hypophysectomized Rats. Nature 145: 388.

(131) Wiltbank, J. N., and Casida, L. E.
1956. Alteration of Ovarian Activity by Hysterectomy. Jour. Anim. Sci. 15: 134.

(132) Wiltbank, J. N., Ingalls, J. E., and Rowden, W. W.
1961a. Effects of Various Forms and Levels of Estrogens Alone or in Combinations with Gonadotrophins on the Estrous Cycle of Beef Heifers. Jour. Anim. Sci. 20: 341.

(133) Wiltbank, J. N., Rothlisberger, J. A., and Zimmerman, D. R.
1961b. Effect of Human Chorionic Gonadotrophin on Maintenance of the Corpus Luteum and Embryonic Survival in the Cow. Jour. Anim. Sci. 20: 827.

(134) Yamauchi, M., and Nakahara, T.
1958. Effects of Uterine Distention on the Estrous Cycle of Cattle. Jap. Jour. Anim. Reprod. 3: 121.

THE LIFESPAN OF CORPORA LUTEA INDUCED DURING THE LUTEAL PHASE OF THE ESTROUS CYCLE IN SWINE.[1]

B. N. Day
Missouri Agricultural Experiment Station, Columbia, Missouri

A more complete understanding of the mechanisms involved in the function and maintenance of the corpus luteum would be very useful in developing more effective methods of controlling the estrous cycle in farm animals. Various types and classes of experimental animals have been utilized in an effort to define the factors causing the persistence of the corpus luteum. In swine, several different experimental approaches have been made in an attempt to extend, experimentally, the functional lifespan of the corpus luteum in non-pregnant animals. Less frequently, attempts have been made to induce premature regression. In general, the procedures used to induce premature regression have not been effective when applied to the corpora lutea of cycling swine. For example, Sammelwitz et al. (7) reported that injected progesterone would cause luteal regression in pregnant swine but not in cycling swine.

The experiment to be discussed here was conducted with non-pregnant gilts. The experimental design included an attempt to cause premature regression by inducing ovulation during the latter part of the luteal phase so that corpora lutea of two different ages would be present on the 14th to 16th day of the estrous cycle. By this procedure, it was possible to determine if the simultaneous regression of the two groups of corpora lutea would occur without respect to age of the luteal tissue. In addition, observations were made on the effectiveness of factors responsible for the development and function of induced corpora lutea in causing the persistence of older corpora lutea present in the same ovary.

Figure 1 presents a summary of the experimental results. In general, when corpora lutea were induced during the luteal phase of the estrous cycle, regression of the spontaneous and induced corpora lutea was not simultaneous. Instead, the induced corpora lutea were maintained for one cycle length following formation, even though regression of the spontaneous corpora lutea occurred without any apparent change in lifespan.

Two separate means of inducing ovulation was used. In one group of animals, ovulation was induced with a single injection of a pituitary FSH preparation. In six of nine animals, the regression and maintenance pattern stated above was exhibited. In two animals having a limited number of induced ovulations and in 1 additional gilt, the induced corpora lutea regressed prior to autopsy. The second method involved the injection of PMS and HCG to induce ovulation. Thirteen animals were examined. Eighty-seven percent of the induced corpora lutea marked during the luteal phase were still present in the ovary in an apparently functional condition at autopsy 20 to 25 days following estrus. These results are presented in more detail by Neill and Day (6).

Figure 1 also presents a summary of the results obtained when corpora lutea were induced in cycling gilts that were subsequently hysterectomized. In these animals, both the spontaneous and induced corpora lutea were maintained for an extended period of time.

Observations were made to determine the functional abilities of the persistent induced corpora lutea. At autopsy, the gross appearance of the induced corpora lutea was not different from that of spontaneous corpora lutea of a comparable age. It was also observed that estrus was not exhibited following regression of spontaneous corpora lutea when induced corpora lutea were present in the ovaries. Studies were made on the progesterone concentration of the induced corpora lutea and, as shown in table 1, the results indicate that the functional abilities of the two sets of corpora lutea were not different prior to regression. The data presented in table 2 represents the progesterone concentration of induced corpora lutea on days 19 to 23 of the estrous cycle or approximately 8 to 10 days following formation. These levels appear to be normal. Progesterone determinations were also made on spontaneous and induced luteal tissue present in one ovary that was surgically removed during the luteal phase of the estrous cycle and these levels were then compared to the progesterone concentration of the corpora lutea present in the opposite ovary of the same animal 3 to 4 days later, which was after regression of the spontaneous corpora lutea. This comparison also suggests that the functional abilities of the spontaneous and induced corpora lutea were not different.

The results presented in tables 2 and 3 provide evidence that the lifespan of the spontaneous corpora lutea was not altered by inducing ovulation. This conclusion is also supported by the findings that both groups of corpora lutea persisted in hysterectomized animals, regressed spontaneous corpora lutea were observed as early as the 18th day of the estrous cycle, and at the time of laparotomy, which was 2 days after the administration of HCG, the spontaneous corpora lutea appeared normal. In contrast to these findings for the pig, Mayer and Klein (4) reported that in rabbits exhibiting 2 superimposed pseudopregnancies, and consequently the ovaries contained 2 sets of corpora lutea, the first generation of corpora lutea involuted rapidly following the formation of the second generation of corpora lutea. However, both generations of corpora lutea were maintained for at least 11 days when estrogen injections were continued following the second mating.

---

[1] Contribution from the Missouri Agricultural Experiment Station.
Journal Series Number 2801.
Approved by the Director.

In many respects, the results obtained in the present study indicate that the lifespan of corpora lutea in cycling swine is determined by a factor intrinsic to the individual gland. However, this does not mean that systemic factors can not also be involved in the regression of cyclic corpora lutea since changes in the receptivity of the corpora lutea to the action of extrinsic luteotrophic or luteolytic factors may be an important part of the complete mechanism. In fact, the maintenance of both spontaneous and induced corpora lutea observed in hysterectomized animals included in the present study suggests the existence of such a mechanism.

If it is assumed that a hypophysial luteotrophic factor is in fact required before persistence of corpora lutea will occur in cycling swine, the results presented here are in agreement with the hypothesis presented by Sammelwitz et al., (7) that the luteotrophic factor in pigs is not released continuously during the luteal phase. Also, by considering that a progestational type of hypophysial blockage normally exists during the luteal phase of the estrous cycle, the results reported here may be compared to those reported by Brinkley et al., (1) on the function of corpora lutea in gilts fed a progestogen to obtain hypophysial blockage. In both experiments it was observed that follicles ovulated by injecting gonadotrophin formed functional corpora lutea. It is felt that these results coupled with those observed in hypophysectomized pigs (du Mesnil du Buisson and Léglise, 5) ascertain that - in contrast to findings in the rat - a continual supply of hypophysial luteotrophic substance is not required for the function of corpora lutea in cycling swine. Similar results have been found in hypophysectomized ewes (Denamur and Mauleon, 2). However, in intact ewes, the lifespan of induced corpora lutea has been reported to be considerably different from that observed in swine. Inskeep et al. (3) found that corpora lutea added to the ovaries as a result of gonadotrophin treatment did not persist after the naturally-formed glands regressed.

In conclusion, it is thought that increased consideration should be given to the possibility that separate - but perhaps related - mechanisms are involved in the persistence of corpora lutea in swine during the luteal phase of the cycle and during pregnancy. Although there is a considerable amount of evidence to support the hypothesis that formation is the only prerequisite to persistence during the luteal phase in swine, there are perhaps not sufficient findings to conclude that a luteotrophic factor is definitely not involved. On the other hand, the available evidence does indicate that a luteotrophic substance of hypophysial origin - or lack of a uterine luteolytic factor that may in fact act through the pituitary gland - does control persistence of the corpus luteum during pregnancy. On this premise, the present approach toward the control of the estrous cycle in swine of extending the luteal phase with orally effective progestogens - or by other designs - does appear to have more likelihood of success than the possible alternative of attempting to decrease the length of the luteal phase with a luteolytic substance in order to synchronize matings.

Literature Cited

(1) Brinkley, H. J., Norton, H. W., and Nalbandov, A. V.
1964. Is Ovulation Alone Sufficient to Cause Formation of Corpora Lutea? Endocrinology 74: 14.

(2) Denamur, Robert, and Mouleon, Pierre.
1963. Contrôle endocrinien de la persistance du corps jaune chez les Ovins. C. R. Acad. Sci. (Par.) 257: 527.

(3) Inskeep, E. K., Oloufa, M. M., Pope, A. L., and Casida, L. E.
1963. Functional Capabilities of Experimentally Induced Corpora Lutea in Ewes. Jour. Anim. Sci. 22: 159.

(4) Mayer, Gaston, and Klein, Marc.
1946. Production d'une nouvelle phase pseudo-gravidique au cours d'une pseudo-grossesse chez la lapine. Evolution des deux générations de corps jaunes. Réactions des cornes utérine et de la glande mammary. Compt. rend. Soc. Biol. 140: 310.

(5) du Mesnil du Buisson, F., and Léglise, P. G.
1963. Effects de l'hypophysectomie sur les corps jaunes de la truie. Résultats préliminaires. C. R. Acad. Sci. (Par.) 257: 261.

(6) Neill, J. D., and Day, B. N.
1964. Relationship of Developmental Stage to Regression of the Corpus Luteum in Swine. Endocrinology 74: 355.

(7) Sammelwitz, P. H., Aldred, J. P., and Nalbandov, A. V.
1961. Mechanisms of Maintenance of Corpora Lutea in Pigs and Rats. Jour. Reprod. Fertil. 2: 387.

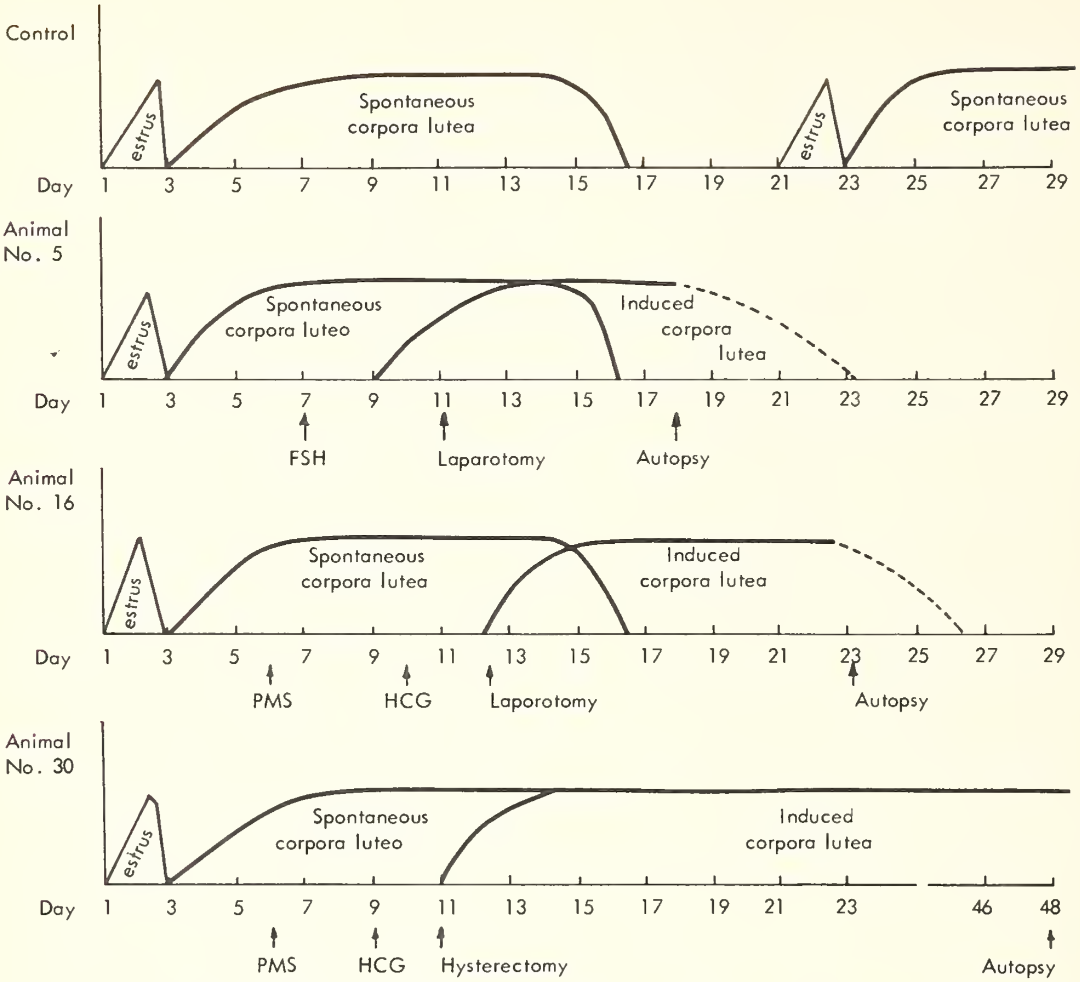

Figure 1. A selected example of each treatment series is depicted for comparison of results with the normal female reproductive pattern of swine. (From Neill and Day, 1964)

Table 1.--Progesterone concentration of spontaneous and induced corpora lutea prior to regression of the spontaneous corpora lutea

	Day of Cycle		No. of Corpora Lutea		Progesterone, µg/gm	
Animal Number	Ovulation Induced	Autopsy	Spontaneous	Induced	Spontaneous Corpora Lutea	Induced Corpora Lutea
41-6	11	14	9	13	44	35
38-2	12	16	11	13	36	38

Table 2.--Progesterone concentration of induced corpora lutea following regression of the spontaneous corpora lutea

	Day of Cycle			
Animal Number	Ovulation Induced	Autopsy	Number of Induced Corpora Lutea	Progesterone, µg/gm of luteal tissue
42-3	11	19	11	30
8-2	13	21	14	38
8-1	13	23	14	41

Table 3.--Within animal comparison of the progesterone concentration of spontaneous and induced corpora lutea before and after regression of the spontaneous corpora lutea

	Animal Number	
	1-5	1-3
Ovulation induced, day	11	12
Number of corpora lutea		
Day 15 or 16		
Spontaneous	5	5
Induced	8	5
Day 19		
Spontaneous	6	10
Induced	9	6
Progesterone, ug/gm of luteal tissue		
Day 15 or 16		
Spontaneous	63	45
Induced	59	63
Day 19		
Spontaneous	9	0
Induced	42	71

FACTORS INFLUENCING THE REGRESSION OF CORPORA LUTEA IN THE EWE, RABBIT AND RAT[1]

H. G. Spies, Kansas Agricultural Experiment Station, Manhattan

Stages in the life of corpora lutea may be divided logically into formation, function and regression. Mechanisms controlling each stage may be independent or overlapping depending on the species. Anderson et al.,(4) have pointed to lack of current evidence to allow a clear distinction between luteinizing hormone (LH) and luteotropic hormone (LTH) in farm animals. Initial LTH release is probably sufficient to form and maintain the corpora lutea for the normal estrous cycle (Aldred et al., 1). Maintenance of corpora lutea for the normal duration of an estrous cycle in hypophysectomized ewes (Denamur and Mauleon, 6) and sows (du Mesnil du Buisson and Léglise, 19) supports this theory. Nalbandov (20) has suggested a second release of luteotropin is initiated due to mechanics of placentation.

Factors responsible for regression of the corpora lutea in most species have not been determined. However, the observed prolongation of functional corpora lutea in several species (review article, Anderson et al. 3) following hysterectomy implicate the uterus. Postulates include a uterine stimulus that blocks LTH release, a uterine provoked luteolytic factor (LLF), or a combination of both.

Ewe

Hysterectomy extends the life of the corpus luteum of the cycling ewe to approximately 160 days (Kiracofe and Spies, 14). The mechanism causing regression of corpora lutea in the hysterectomized ewe appears to differ from the mechanism of cycling ewes. Corpora lutea induced following natural ovulation in the hysterectomized ewe do not regress synchronously with naturally formed corpora lutea as reported for the cycling ewe (Inskeep et al., 10), but persist for about 160 days from induction (Kiracofe and Spies, unpublished manuscripts). Asynchronous regression of induced and naturally formed corpora lutea in hysterectomized ewes may indicate the presence of a LLF in the intact ewe. The necessity of the pituitary to maintain corpora lutea in hysterectomized ewes (Denamur and Mauleon, 6) beyond 20 days suggests that asynchronous regression of induced and naturally formed corpora lutea may result from a difference in competence of different-aged luteal tissues to gonadotropin stimulation. Differences in response of sheep luteal tissue to a given level of PMS in vitro support this hypothesis (Legault-Demare et al., 16).

Thus far attempts to isolate a uterine LLF have failed. Ether extracts of uteri removed from ewes during estrus to 4 to 7 days post estrus did not influence the weight or histology of the corpora lutea when injected into hysterectomized ewes during 11 - 19 days following ovulation (Kiracofe et al., 14). Freeze-dried extracts of uteri removed on days 12 to 14 following ovulation or on the day of estrus have also failed to regress the corpora lutea when injected into cyclic or pregnant ewes hysterectomized prior to injections, or when injected into normal 25-day pregnant ewes (Kiracofe and Spies, unpublished manuscript). These results must be used cautiously as extraction procedure; amount of extract or type of test animal may be involved in the negative response. A small percentage of hysterectomized ewes receiving uterine extracts possessed small accessory corpora lutea at slaughter. None was observed at preinjection laporatomy or in noninjected hysterectomized controls. It is not known if gonadotropin stimulation resulted from uterine extracts.

Bilateral ligation of the mid-uterine arteries and veins prolonged the life of cyclic corpora lutea in ewes, while unilateral ligations had no effect (Kiracofe et al., 13). A species difference may exist between ewes, sows and guinea pigs (Rowlands, 24) in regard to a localized effect of the uterus on the corpora lutea (du Mesnil du Buisson, 18). The uterine effect on the luteal regression mechanism in the ewe probably occurs rapidly. Stormshak et al. (25) indicated that a decrease in corpora lutea weight and progesterone concentration had not occurred by day 14 in the cycling ewe. Also, hysterectomy of ewes as late as day 16 post ovulation resulted in maintenance of the corpora lutea (Kiracofe and Spies, 15). This agrees with results in the guinea pig where hysterectomy on day 15 of the cycle maintained the corpora lutea (Rowland, 23). The mechanism appears to act somewhat earlier in the pig since hysterectomy after days 16 - 18 of the 21-day cycle resulted in regression of the corpora lutea and new ovulation (Anderson et al., 2). In addition du Mesnil du Buisson (18) reported that unilateral regression of corpora lutea in the pig was evoked between day 14 and 16 post ovulation. Although corpora lutea are maintained in ewes hysterectomized before day 16 of the cycle, the corpora lutea are smaller when the uterus is removed after day 14 (Kiracofe and Spies, 15). A slower rate of luteal regression has been reported in hypophysectomized rats than in intact animals

---

[1] Department of Animal Husbandry, Contribution No. 314, Kansas Agr. Expt. Station, Manhattan

(Greep, 8). Also du Mesnil du Buisson and Léglise (19) observed decreased luteal regression in hypophysectomized, hysterectomized sows compared with intact females, suggesting a possible uterine-pituitary interaction.

Rabbit

Induced corpora lutea of the cycling gilt regress asynchronously (Neill and Day, 21) as do corpora lutea of hysterectomized ewes, in contrast to the synchronous regression of corpora lutea in cycling ewes (Inskeep et al., 10). Rabbits differ from both ewes and pigs as induction of a second group of corpora lutea in pituitary intact pseudopregnant rabbits will cause rapid regression of the initial group of corpora lutea at any stage of pseudopregnancy beyond day 4 (Coon and Spies, 5). HCG, NIH-LH or NIH-FSH plus LH produced new ovulations and involution of the initial set of corpora lutea. Four-day-old corpora lutea did not regress when does were treated with HCG, but histologically appeared small, avascular, and poorly developed. Corpora lutea induced at day 3 of pseudopregnancy and initial corpora lutea regress synchronously; however, regression occurred approximately 18 days after the second ovulation. These results differ from those reported for the cycling ewe.

Estrogen was reported to be luteotropic in the rabbit (Robson, 22; Hammond Jr. and Robson, 9). LH treatment after day 4 post ovulation in the pituitary intact rabbit appears to block estrogen, thus causing corpora lutea regression. Estrone administered concurrently with LH prevents regression of the initially formed corpora lutea (Coon and Spies, 5). A second ovulation may be provoked and the two groups of corpora lutea regress asynchronously about day 18 post respective ovulations. Observations on corpora lutea induced in the hysterectomized-pseudo-pregnant rabbit are similar to observed results in the uterine-intact rabbit except the second group of corpora lutea persists 25-28 days (Spies and Coon, unpublished manuscript). Kilpatrick et al. (12) reported that LH was luteotropic in hypophysectomized does. The contrasting effect of LH in pituitary intact does, compared with hypophysectomized does, suggests LH may block estrogen via a pituitary pathway.

Rat

verett (7) has suggested that the same neurohumoral factors from the hypothalmus that stimulate LH discharge inhibit prolactin release in the rat. Greep (8) has suggested LH has a luteolytic effect in the hypophysectomized rat, and although this work has not been confirmed, de Jongh and Wolthuis (11) indicated Rothchild has recently observed that exogenous LH injected into rats with an extra pituitary placed in the renal capsule caused decreased size and progesterone production. Melampy et al. (17) has suggested their work with hysterectomized-pseudopregnant rats provided evidence of an uterine-luteolytic mechanism. Spies and Kiracofe (unpublished manuscript) have not been able to identify a uterine LLF from rat uterine extracts or parabiotic rats. Freeze-dried uterine extracts from estrual rats did not shorten the vaginal cycle of hysterectomized-pseudopregnant rats. Parabiotic union of ovariectomized females with intact-pseudopregnant or hysterectomized-pseudopregnant rats increased vaginal cornification of the ovarian intact partner, probably as a result of increased estrogen output. However, the presence of India ink-marked, histologically functional corpora lutea suggests the life of the corpora lutea was not shortened. Vaginal cycles of normal intact cycling females were not affected when paired with hysterectomized-pseudopregnant females, nor were any effects on ovarian histology of the hysterectomized-pseudopregnant partner observed.

Literature Cited

(1) Aldred, J. P., Sammelwitz, P. H., and Nalbandov, A. V.
1961. Mechanisms of Formation of Corpora Lutea in Guinea Pigs. Jour. Reprod. and Fertil. 2: 394.

(2) Anderson, L. L., Butcher, R. L., and Melampy, R. M.
1963. Uterus and Occurrence of Oestrus in Pigs. Nature 198: 311.

(3) Anderson, L. L., Bowerman, A. M., and Melampy, R. M.
1963. Neuro-Utero-Ovarian Relationships. Advances in Neuroendocrinology. Univ. Ill. Press, Urbana P. 345-373.

(4) Anderson, L. L., Schultz, J. R., and Melampy, R. M.
1963. Pharmacological Control of Ovarian Function and Estrus in Domestic Animals. Sixth Anim. Reproductive Symposium, Corvallis, Oreg. W. H. Freeman and Co., PP. 171-219.

(5) Coon, L. L., and Spies, H. G.
1964. Anti-Luteotrophic Effect of LH on Rabbit Corpora Lutea. Jour. Anim. Sci. 23. (Abstr.).

(6) Denamur, R., and Mauleon, P.
1963. Controle endocrinien da la persistance du corps jaune chez les ovins. C. R. Acad. Sci. 257: 527.

(7) Everett, J. W.
1956. Functional Corpora Lutea Maintained for Months by Autografts of Rat Hypophysis. Endocrinology 58: 786.

(8) Greep, R. O.
1938. The Effect of Gonadotropic Hormones on the Persisting Corpora Lutea in Hypophysectomized Rats. Endocrinology 23: 154.

(9) Hammond, J. Jr., and Robson, J. M.
1951. Local Maintenance of the Rabbit Corpus Luteum with Oestrogen. Endocrinology 49: 384.

(10) Inskeep, E. K., Oloufa, M. M., Pope, A. L., and Casida, L. E.
1963. Functional Capabilities of Experimentally Induced Corpora Lutea in Ewes. Jour. Anim. Sci. 22: 159.

(11) de Jongh, S. E., and Wolthuis, O. L.
1964. Factors Determining Cessation of Corpora Lutea Function: The Possible Role of Oestradiol and Progestrone. Acta. Endo. Supplementum 90: 125.

(12) Kilpatrick, R., Armstrong, D. T., and Greep, R. O.
1964. Maintenance of the Corpus Luteum by Gonadotrophins in the Hypophysectomized Rabbit. Endocrinology 74: 453.

(13) Kiracofe, G. H., Spies, H. G., and Gier, H. T.
1963. Effect of Uterine Extracts and Ligations in Hysterectomized Ewes. Jour. Anim. Sci. 22:862 (Abstr.).

(14) Kiracofe, G. H., and Spies, H. G.
1963. Length of Corpora Lutea Maintenance in Hysterectomized Ewes. Jour. Anim. Sci. 22: 862 (Abstr.).

(15) Kiracofe, G. H., and Spies, H. G.
1964. Late Luteal Hysterectomy and Uterine Extracts in Ewes. Jour. Anim. Sci. 23: (Abstr.).

(16) Legoult-Demare, J., Mauleon, P., et Saurez-Soto, M.
1960. Etude de l'activite biologue in vitro des hormones gonadotropes II. Stimulation in vitro du corps jaune de brebis par l'hormone gonadotrope serique de jument gravide (PMSG). Acta. Endocrinology 34: 163.

(17) Melampy, R. M., Anderson, L. L., and Kragt, C. L.
1964. Uterus and Life Span of Rat Corpora Lutea. Endocrinology 74: 501.

(18) du Mesnil du Buisson, F.
1961. Regression unelaterale des corpa jaines Apres hysterectomie patielle chez la truie. Ann. Biol. Anim. Bioch. and Biophys. 1: 105.

(19) du Mesnil du Buisson, F., and Léglise, P.C.
1963. Effects del' hypophysectomie sur la corps jaune chez la truie. C. R. Acad. Sci. 257: 261.

(20) Nalbandov, A. V.
1961. Comparative Physiology and Endocrinology of Domestic Animals. Recent Prog. Hormone Res. 17: 119.

(21) Neill, J. D., and Day, B. N.
1964. Relationship of Developmental Stage to Regression of the Corpora Lutea in Swine. Endocrinology 74: 355.

(22) Robson, J. M.
1937. Maintenance by Oestrin of the Luteal Function in Hypophysectomized Rabbits. Jour. Physiol. 90: 435.

(23) Rowlands, I. W.
1961. Effect of Hysterectomy at Different Stages in the Life Cycle of the Corpus Luteum in the Guinea Pig. Jour. Reprod. and Fertil. 2: 341.

(24) Rowlands, I. W.
1962. The effects of Oestrogens, Prolactin and Hypophysectomy on the Corpora Lutea and Vagina of Hysterectomized Guinea Pigs. Endocrinology 24: 105.

(25) Stormshak, F., Inskeep, E. K., Lynn, J. E., Pope, A. L., and Casida, L. E.
1963. Progesterone Levels in Corpora Lutea and Ovarian Effluent Blood of the Ewe. Jour. Anim. Sci. 22: 1021.

DR. SPIES: I would like to have a definition of the term "luteolytic factor." Are we simply talking about a "factor" which blocks LTH or does the "factor" cause regression of the corpus luteum in the presence of luteotropin?

DR. MELAMPY: Dr. Day, do you wish to answer this question?

DR. DAY: I don't believe I would call it luteolytic. Maybe use of the term has been confined to a direct effect. I think it could be some factor which is anti-luteotropic but whether this is gonadotropic or not I do not know. But it could act, let us say, through the pituitary.

DR. SPIES: I wonder if we are all consistent in the use of this term. Are we all using it in this light or do we have differences in the way different workers are using the term as results are reported in the literature?

DR. MELAMPY: It is realized that there are many unsolved problems in the field of corpus luteum physiology. This is particularly true as experimental results become available from investigations dealing with different species. Among these problems are what physiologic factor(s) are involved in the formation of the corpus luteum? Is this associated with LH activity or is it dependent upon a specific luteotropin? Also, what mechanisms are involved in the maintenance of progesterone secretion? What are the functional and morphologic changes associated with the initiation of luteal regression? What is the role of the uterus in luteal function? The effect of hysterectomy on ovarian function has been studied in several species. Total hysterectomy has been observed to cause persistence of corpora lutea for a period of time approaching or exceeding the length of gestation in the guinea pig, ewe, sow, and cow, but hysterectomy of the ferret and opossum has no apparent effect on ovarian function or mating behavior. The effects observed following uterus removal in the monkey and woman are not well defined. This may be due to a lack of systematically controlled observations over a period of time. Hysterectomy in the unmated rat and rabbit has no apparent immediate effect on ovarian activity, whereas hysterectomy of pregnant and pseudopregnant females of these species results in postponement of luteal regression. It is to be noted that hysterectomy leads eventually to ovarian degeneration in several species. The physiologic basis of uterine regulation of luteal persistence is still obscure. Results from experimental work on the heifer, gilt, ewe, and guinea pig indicate the absence of the uterus produces a physiologic state which is compatible with persistence of functional corpora lutea. Furthermore non-specific portions of the uterine horns are adequate to initiate the onset of luteal regression during the estrous cycle of the unmated female of these species. The occurrence of estrous cycles has been observed in gilts and guinea pigs following uterine autotransplants. It is concluded that a functional endometrium appears to be necessary for the initiation of luteal regression in gilts and guinea pigs with intact uteri as well as in animals with autotransplanted uteri. These observations suggest that a non-neural uterine stimulus is possibly involved in regulating the life span of the corpus luteum in certain species. It is possible that the uterus of the non-pregnant female maintains a positive inhibition of pituitary luteotropin release. This may be either hormonal or neuroendocrine in nature. In pregnancy and following hysterectomy this inhibition is lacking, hence, the persistence of functional corpora lutea. It is suggested that the uterus functions as a pacemaker in determining the longevity of corpora lutea and, as a result, regulates the initiation of luteal regression and hence the occurrence of cycles.

Dr. Anderson, do you want to comment on any of these questions?

DR. ANDERSON: With regard to Dr. Spies' question about the term "luteolytic factor", I think we may refer to luteolytic action, which in the case of the uterus could be the result of a particular uterine physiologic state at a certain stage of the estrous cycle rather than attributing the luteolysis to a "factor" of uterine origin.

DR. MELAMPY: I would like to ask Dr. Nalbandov a question. What do you think about the significance of LH and progesterone production by the corpus luteum?

DR. NALBANDOV: First, may I just call your attention to the fact that the oft-quoted work of Greep which dates back to the 1930's, that LH is luteolytic in rats, has been withdrawn by Greep in the publication called "Control of Ovulation," in which he states that he has repeated this work within the last few years with more purified LH preparations and has not found it to be luteolytic. I noticed that several people have quoted his work, the earlier work, without quoting the correction which he has made in subsequent years.

In the same volume, "Control of Ovulation," some work by Parlow is quoted which was later confirmed by him, (Recent Progress in Hormone Research, 1961) that LH is luteotropic in the rat, but in an entirely different sense. If you hypophysectomize the rat and treat it with LH, then, instead of producing progesterone, the rat now produces estrogen in sufficient quantities to cause vaginal cornification which persists for prolonged periods of time. This occurs many months after hypophysectomy. These hormones are apparently potentially able to affect tissue which was initially intended to produce progesterone in such a way as to make it secrete estrogen. We have been very careful in our own work not to speak of luteotropins but of luteotropic factors, and this is because I personally have no idea what luteotropin will turn out to be. My suspicion is that it may be LH, but it may equally well turn out to be an as yet unidentified factor. That is the best answer I can give you at the time.

DR. MELAMPY: Thank you. An important contribution by T. Eto, H. Masuda, Y. Shzuki, and T. Hosi (Japanese Journal of Animal Reproduction 8:34-40, 1962) should be mentioned at this time. These investigators determined the progesterone and pregn-4-ene-20α-ol-3-one concentrations in rat ovarian venous blood at different stages of the reproductive cycle. It was found that progesterone concentration increases to a maximum during the afternoon of proestrum and reaches approximately 110 mcg./100 ml. Following this peak it declines but shows an increase again in early diestrum. Pregn-4-ene-20α-ol-3-one is secreted throughout the estrous cycle and attains a maximum value of approximately 240 mcg./100 ml. of blood at early diestrum. During gestation the progesterone concentration is high on day 4 and reaches a maximum value on day 15. It is low, however, immediately before and after parturition. On the other hand pregn-4-ene-20α-ol-3-one concentration is lower during the first half of gestation than during the estrous cycle, but at day 15 it is approximately 260 mcg./100 ml. On day 8 of lactation blood progesterone concentration is high and it is higher in females with litters of 6 pups than in those with 2. Ovaries of hypophysectomized rats bearing renal pituitary grafts secrete a large amount of progesterone but little pregn-4-ene-20 α-3-one.

DR. DZIUK: Before we get on to the slide, I would like to ask two questions that may be related. (1) How does unilateral regression of the corpora lutea occur in the pig if the central nervous system is involved? And (2) Does the number of embryos influence the corpus luteum number so that we perhaps have been looking at corpora lutea counts and embryo counts all this time and assuming wrongly a certain proportion of embryonal deaths? Are we actually looking at a cause and effect in which the number of embryos may influence the number of corpora lutea present?

And now I would like to show the slide. I would like to suggest that perhaps LH, as we think of it, causes ovulation but does not necessarily cause luteinization (table 1).

These animals were treated with high levels of progesterone for nine days and then, since no further treatment is applied, wouldn't ovulate spontaneously for at least 10 days, and most of them wouldn't ovulate for about 20 days (Dziuk and Baker, 1962, Jour. Anim. Sci. 21: 697). On the 6th day after the last progesterone feeding we performed a laparotomy and punctured the follicles. All gilts were slaughtered 7 days after laparotomy. The ovulating "hormone" here was a scalpel or needle. The "C" represents a triangular cut in the follicle wall and the "S" is a puncture with scarifications. We thought we were not getting enough bleeding by just puncturing, which is indicated by the letter "P". Actually this is a little bit reversed. We started out puncturing the follicles and letting the fluid out. It turned out, as you can see, in gilts 1 through 3, that we got about the same number of follicles at slaughter as we had at laparotomy and no corpora lutea were formed when follicles were punctured. So, we thought the follicles were healing over. Later we cut or scarified follicles on one ovary and punctured follicles on the opposite ovary in gilts 4 through 9. At autopsy we observed corpora lutea on the cut or scarified side but few corpora lutea on the ovaries whose follicles had been punctured. As it turns out, the size of the hole seemed to make a considerable difference in whether we got corpora lutea formed or not. This suggests to me, that LH causes ovulation but has little to do with corpora lutea formation, and that the corpus luteum would form if the fluid was just released from the follicle.

DR. MELAMPY: Do you want to comment on that?

DR. ANDERSON: The unilateral regression of corpora lutea in gilts on the ovary on the side with a nongravid uterine fragment has been observed under experimental conditions by du Mesnil du Buisson (Compt. Rend. Acad. Sci. 253:727, 1961) and by Rathmacher and Anderson (Jour. Anim. Sci. 22:1139, 1963). This apparent luteolytic action of the uterine fragment may be a local effect. A local humoral or neuro-humoral effect may be present from the nongravid uterine fragment on the adjacent ovary. Also, alterations in hemodynamic relationships between the uterus and ovary may contribute to this phenomenon. Gilts with uterine autotransplants exhibit normal estrous cycles which would rule out at least major neural pathways for the uterine action that results in luteal regression. However, vasomotor nerves would be present in the autotransplanted uterus.

DR. MELAMPY: Dr. Casida, would you like to comment on the triangular ovulations?

DR. CASIDA: I am going to comment on unilateral regression.

One point of view on the cause of regression of corpora at the end of the cycle, or at least at the end of pseudopregnancy in the rat, is that gonadotropins, presumably FSH and LH, eventually come into the picture and interfere with the action of luteotropin for maintenance purposes. I believe this is the point of view of Dr. Rothchild. This raises the question as to whether a substance may not be produced in the uterus which is effective locally and which plays a role with the gonadotropin in bringing about regression of corpora. Perhaps sensitizing the corpora lutea to the action of gonadotropins to bring about the regression.

It seemed to us that the rabbit might be a very good animal in which to check this point

Table 1.--Corpus luteum formation after follicle puncturing in the pig

	Left Ovary				Right Ovary			
	Laparotomy		Autopsy		Laparotomy		Autopsy	
Gilt	Follicles no.	Type of cut[1]	Corpora lutea no.	Follicles no.	Follicles no.	Type of cut	Corpora lutea no.	Follicles no.
1	9	P	0	5	5	P	0	3
2	10	P	0	9	5	P	0	2
3	6	P	0	5	9	P	0	8
4	5	S	5	1	8	P	5	1
5	9	S	6	0	11	P	7	0
6	6	S	8	0	8	P	6	0
7	4	C	3	0	9	P	0	6
8	10	C	6	0	6	P	0	8
9	6	C	6	1	10	P	0	4
10	6	C	6	0	7	S	4	1
11	8	C	5	6	6	S	0	10
12	7	C	0	1	7	S	0	8
13	11	C	11	0	7	C	7	0
14	9	C	9	0	5	C	5	0
15	12	C	12	0	5	C	8	0
16	9	C	5	5	13	C	2	6
17	12	C	6	4	4	C	4	1
18	6	C	4	2	8	C	7	2
19	7	C	4	2	8	C	4	3

[1] P - Puncture follicle with suturing needle.
S - Puncture follicle and rub inside of follicle with needle.
C - Cut follicle with "V" shaped incision 4 mm. each side of V.

where the injection of LH, as Dr. Spies indicated, causes regression of the corpora lutea. If there is a hysterectomy of these animals, will it work? That is, can injected LH bring about regression of corpora lutea in the hysterectomized animals? Regression did occur in hysterectomized animals and we did not get evidence for a uterine factor which could act locally between the gonadotropins and the corpus to bring about regression.

I might raise a question regarding your statement that estrogen maintains corpora lutea, or is it luteotropic in the hypophysectomized rat? Am I quoting you correctly on that? I have been unable to substantiate this point. There is evidence, I believe, that estrogen in the hypophysectomized rat will cause a marked change in the amount of granulosa tissue and in the number of follicles that show marked hypertrophy, and it will also affect, perhaps, the ability of PMS or HCG to bring about excessive stimulation or even some luteinization, but does this mean that estrogen is luteotropic in the hypophysectomized animals? I doubt that it does. It may be a matter of definition. What do we mean by luteotropin? Does a substance which will synergize the follicle-stimulating-luteinizing action of something like HCG really qualify?

DR. MELAMPY: With regard to Dr. Casida's comment pertaining to the effect of estrogen on the rat ovary, Bradbury (Endocrinology 68:115-120, 1961) observed ovaries following application of crystalline estradiol or stilbestrol to one ovary of the immature rat leaving the other ovary untreated. It was found that the estrogen-treated ovary exhibited several unilaterally differentiated responses: (1) a greater increase in weight, (2) an increased responsiveness to endogenous and exogenous gonadotropins. In control experiments crystalline testosterone or progesterone was applied to one ovary with, or without, estradiol being placed on the other ovary. These experiments demonstrate that estrogen has a local stimulatory effect within the ovary as well as a systemic effect via the pituitary. Pencharz (Science 91:554, 1940) and Williams (Nature 145: 338, 1940) observed that stilbestrol pellets implanted into rats at time of hypophysectomy not only increased ovarian weights but also made the ovaries more responsive to exogenous gonadotropins. Stilbestrol has been used in hypophysectomized and intact rats to augment ovarian response to gonadotropins by Payne and Runser (Endocrinology 65:383, 1959) and Meyer and Bradbury (Endocrinology 66:121, 1960). The physiologic basis of the observed stimulating action of estrogen on ovarian function is unknown at present.

I would like to briefly mention some preliminary observations we have made relative to the physiologic state of the uterus to luteal function in the rat. In this experiment it was found that the duration of pseudopregnancy in intact rats (Holtzman strain) is 14.4±0.44 days. This is extended to 22.1±0.84 days following hysterectomy. In parabiotic rats, with one animal of each pair having an intact uterus and the other hysterectomized, the length of the pseudopregnancy in the hysterectomized rat was significantly reduced when the parabiotic union was made either before or after induction of pseudopregnancy. Homotransplantation of an estrous uterus from one animal into the abdominal cavity of its pseudopregnant littermate sib resulted in a significant reduction in the length of diestrum when the transplant remained viable. It was also observed that autotransplants of ovaries into the uterine horns of estrous rats reduced the duration of pseudopregnancy to 11.5±0.47 days as compared with 14.4±0.44 days in the control groups ($P < 0.01$).

I would like to ask Dr. Hansel if he would like to comment on LH and progesterone synthesis in luteal tissue.

DR. DONALDSON: I think that what I am supposed to say is we think LH is luteotropic in the cow. The evidence for this is based upon overcoming oxytocin inhibition with an LH preparation which does not contain any FSH and the ability to do this with HCG, but not with HCG that has been incubated with 6-M-urea to destroy the LH component. The ability of a crude pituitary preparation to overcome oxytocin inhibition and the inability of bovine prolactin or ovine prolactin to do so are shown in the data on this slide. What we did was to take Holstein heifers, treat them with oxytocin, and take the corpus luteum by surgery either on day 4 or day 7. As you can see, on day 4, oxytocin did not affect luteal function as measured by size, progesterone content per gram, or total progesterone. Oxytocin reduced the total progesterone, progesterone per gram, and the corpus luteum weight on day 7. Now, if we gave HCG on top of this on day 4, nothing happened, but at day 7 we got an increase in all of these parameters. Bovine LH did the same; prolactin did not and urea incubated HCG did not.

To support this claim, we collected pituitaries on day 0, day 4, and day 7; 10 pituitaries on each of these days, half of which were treated with oxytocin and half of which were not. The data in this slide show that if a single injection of oxytocin is given as soon as we detect an animal in heat and then she is killed six hours after she is detected in heat, the total gonadotropin content of the pituitary is halved. This supports the observation made some years ago that oxytocin given during estrus will hasten ovulation.

At day 4 oxytocin did nothing to the pituitary gonadotropin content, but at day 7 oxytocin halved pituitary gonadotropin content. The levels at day 0 were approximately 3 units/mg. of dried anterior pituitary weight in the controls, and 1.5 units/mg. in oxytocin-treated heifers. At day 4 the levels were about 10 in each, and at day 7 the levels had increased in the controls to about 19, and oxytocin reduced them to about 8.

The most striking bit of evidence, I think, was when we did a correlation on the pituitary gonadotropin levels and the progesterone contents of the corpora. In control animals this correlation was large and negative, -0.75, but in the oxytocin-treated animals at day 7 the correlation was large, 0.96 and positive. This seemed to indicate at least in the oxytocin-treated animals, that pituitary level indicated plasma level and that it was the plasma levels of gonadotropin that were controlling and limiting corpus luteum function, as demonstrated by progesterone content or luteal weight. We believe that it is probably LH in this total gonadotropin fraction that is responsible for this result. We have not had very much success with a specific LH bioassay. The bioassay we used on these pituitaries was P^{32} uptake on the chick testis. It worked very well. We got quite good confidence limits and generally it was very satisfactory. I think this is fairly good evidence that LH is luteotropic in the bovine.

DR. HANSEL: May I add just two points to that statement? Perhaps the point about the urea-incubated HCG was not made clear. Incubation with urea is said to destroy the LH component of HCG and to destroy its ability to stimulate progesterone production of the corpora after the incubation. One other point that is perhaps worth presenting concerns the fact that one can produce larger than normal corpora by LH.

DR. DONALDSON: This shows a big CL that was grown with bovine LH. It weighed 9.5 grams at 7 days.

DR. MELAMPY: Was it solid?

DR. HANSEL: Yes. That one was.

Now, added to these data are those of Armstrong, Black, and Cone (Fed. Proc. 23: 462, 1964) which you see here in incomplete form. These are the results of their incubation studies in which they added the NIH bovine LH preparation to luteal tissue slices obtained from cows at various stages of the cycle: 0, 5, 6, and 9 et cetera. You will note that when they add LH they get a progesterone stimulation until day 17. Then, the stimulation cuts out, apparently rather abruptly, on day 18.

It seems to me, when you put together the in vivo results that Donaldson has talked about, with these in vitro results it is strongly indicated that LH is luteotropic. The only loophole that is left is that there could be some synergistic effect of FSH and LH as a result of the FSH contamination in the so-called purified LH preparation.

DR. NALBANDOV: I would just like to point out that I think these data are very nice but we should not confuse luteotropic effect, that is, the ability of the hormone to keep a corpus alive and functioning, with the ability of a hormone to make the corpus secrete progesterone in vitro. I think these effects are entirely different and we may find that they depend on entirely different systems.

Would you like to comment on that?

DR. HANSEL: I am afraid I haven't much to add, but as I get the remark it is suggested that there is a difference between the ability of a compound to cause progesterone production in vitro, and its possible in vivo effect. To be sure, Armstrong's data are subject to this criticism, but the data that Donaldson cited are all in vivo data. So far as I know this is the only in vivo system for testing a luteotropic effect. It is the only way in which we can inhibit a corpus luteum, with the exception of estrogen injections, as was pointed out a while ago. These are in vivo results.

DR. NALBANDOV: In pituitary intact animals, however?

DR. HANSEL: Right.

DR. MELAMPY: Thank you. Any other questions?

DR. WILTBANK: I would like to mention that we have preliminary information at Fort Robinson on a limited number of animals. We are doing an experiment now in which we have been able to cause regression of the corpus luteum with around 640 mcg. of estrogen given daily. Then we have tried to reverse this effect with FSH and LH and we can get a reversal of the regression of the corpus luteum by giving these hormones, which, again, would indicate then that the LH is luteotropic and we can get it with just the LH alone.

DR. HANSEL: May I ask what kind of an LH preparation?

DR. WILTBANK: We are just using Armour's LH at the present time, so there was some FSH contamination in it.

DR. ANDERSON: In the hypophysectomized-hysterectomized gilt, corpora lutea are maintained from days 12 to 20 with Armour's LH. Corpora lutea are not maintained as well during this period in the hypophysectomized pig in which the uterus remains intact. The luteotropic effect of LH in the hypophysectomized-hysterectomized gilt is apparent, for in the absence of exogenous hormone support in these animals complete regression of corpora lutea and follicles occurs during this period.

DR. SPIES: I have two questions. One to Dr. Melampy and one to Dr. Wiltbank.

I will direct the first one to you, Jim. Since you have been able to produce regression of the fully formed corpus luteum in the cow with estrogen, would you say that this species differs from the pig and the ewe in that the cow corpus luteum depends on the pituitary throughout the cycle?

DR. WILTBANK: I think the indications that we have would say that this is true, that the bovine may be different than the ewe or the sow in this respect.

DR. SPIES: Dr. Melampy, I wish to ask you to comment on the statement that in the parabiotic rats you observed a shortening of the cycle. Was this observation based on the vaginal cycle or histological study of the ovary?

DR. MELAMPY: We observed the cycling effect and also from the preparations we made histological observations on them, and the corpora lutea in some of these were in a state of regression.

Now there is one person here who has been working in this area and I would like to call on Dr. W. D. Foote because he has been doing some work on gonadotropic activity in the bovine pituitary and I think it is quite fitting that we should hear about this at this time.

DR. FOOTE: The work we have been doing at Nevada has not been directed toward the function of gonadotropins relative to their association with corpus luteum maintenance.

We have been trying to get basic information on what the pituitary is doing, in terms of FSH and LH activities in untreated animals. We have been working both with the beef heifer and to some extent the post-partum beef cow.

I think our results are interesting in view of the comments that were made regarding LH during the cycle. We have nothing that shows whether or not it has the luteotropic activity. However, the trend that we obtained for LH content of the pituitary gland is somewhat interesting. The LH content of pituitary glands taken at day 0, 2, 8, 16, and 19 of the estrual cycle showed the highest LH content to be present at day 16. The lowest content was at day 0. From day 0 there seemed to be a steady increase through day 8, up to the high level at day 16. Then, for some reason, we found a decrease in LH activity between day 16 and day 19, which we could, I guess, consider to be related to the time the corpus luteum might normally start to regress and presumably decrease in progesterone production. We found a further decrease between day 19 and day 0. The FSH material is not quite complete, but it appears to follow somewhat the same trend during the cycle as the LH.

In the post-partum animals, we recovered pituitaries on day 5, day 17, and day 30, after calving, using only glands from cows which had not ovulated at the time of slaughter. The tendency here was for the lowest level of LH to be at day 5, an increase at day 17, and then a more marked increase between day 17 and day 30. This indicates, I suppose, that during this recovery period, or whatever the nature is of the post-partum anestrual period, the cow is beginning to build up again the level of LH. This, of course, refers to pituitary content. It does not tell us what is actually present in the blood. Here, again, the FSH picture is not complete, but it does not seem to follow the LH trend as it did in the cycling heifers. In fact, our results suggest that an inverse relationship may exist between LH and FSH levels at this reproductive stage.

DR. MELAMPY: Thank you very much. I want to thank Dr. Anderson, Dr. Day, and Dr. Spies for participating and also the members in attendance at the symposium.

We stand adjourned until tomorrow morning.

ROLES OF HYPOTHALAMUS AND HYPOPHYSIS IN THE CONTROL OF THE ESTROUS CYCLE

A. V. Nalbandov
Department of Animal Science
University of Illinois, Urbana, Illinois

The interrelation between the hypothalamus and the anterior pituitary gland has been firmly established. It is not the intention of this report to review the literature on this extensive subject. Such reviews are available in "Advances in Neuroendocrinology" (Nalbandov, 2), and since the time of this publication many papers on this subject have appeared in current journals. For the purposes of the present discussion a few fundamental facts will be restated, but the major part of my presentation will deal with my own views of the research areas which should be of special interest to animal scientists concerned with phenomena of reproduction.

A majority of endocrinologists will agree on the following basic points. The adenohypophysis is still considered to be the site of synthesis of all the hormones usually ascribed to it. The neurohypophysial hormones are known to be synthetized in the hypothalamic nuclei; they are present in the median eminence but the neural lobe is considered to be only the storage place for oxytocin, vasopressin in mammals, and oxytocin and vasotocin in birds. The kind of hormones synthesized by the adenohypophysis depend on the proximity of that lobe to the hypothalamus since separation of these two structures by a barrier or by distance causes cessation of the production of the usual hypophysial hormones. In the rat, a profound shift from the secretion of the usual hormonal spectrum to only one hormone - prolactin - occurs if the pituitary gland is removed from the sella and implanted, for instance, in the kidney. This implies that the hypothalamus secretes substances which may direct the synthesis of hormonal precursors into the specific hormones which are usually associated with the adenohypophysis.

The physiological dependence of the adenohypophysis on the hypothalamus is further illustrated by the finding that only those pieces of the anterior lobe autotransplanted into the third ventricle which abut the hypothalamus retain their histological and apparently their physiological integrity. Those portions, too far removed from this so-called hypophysiotrophic area of the hypothalamus, become cytologically abnormal and no longer possess the normal population of hypophysial cells.

It is well established that the hypothalamus also produces so-called "releasing factors" which reach the adenohypophysis via the portal system and which can cause the release of previously synthesized hypophysial hormones into the peripheral circulation.

Specific releasing factors (RFs have been demonstrated either in vivo or in in vitro systems (or in both) for ACTH (CRF), TSH (TRF), LH (LHRF or LRF), somatotrophin (SRF), and FSH (FSHRF). In the rat it has been shown that the hypothalamus produces an inhibiting factor, (not specifically isolated), which prevents release, and perhaps synthesis, of prolactin. Those releasing factors which have been best characterized chemically are small polypeptides which resist heating and can thus be separated from hypophysial trophic hormones. The advent of sephadex column has greatly facilitated research on releasing factors and has made their identification and separation from the hypophysial hormones and nonspecific proteins possible.

It remains unclear whether the RFs must signal the release of all hypophysial hormones needed in the routine day-to-day requirements of an animal, or whether they come into play only when superquantities of such hormones are required as in the case of ACTH to meet stress situations, or in the case of LH, to cause ovulation. The fact that releasing factors have been found for FSH and somatotrophin suggest that release of all hormone quantities may require a hypothalamic impetus in the form of releasing factors.

All of these facts are based on work done in rats but in most of the published work the releasing factors were obtained from hypothalamic fragments obtained from cattle, sheep and, in a few cases, from pigs. As far as I know, no work on the effect of releasing factors on domestic animals, other than the chicken, is in progress. This is due to the fact that hypothalamic fragments are small and difficult to accumulate in sufficiently large quantities to be tested on the larger domestic animals which, for the time being, are probably unsuitable even for pilot studies involving systematic injection. Nevertheless such studies should be undertaken using special techniques to make certain that adequate quantities of the releasing factor to be studied reach their target tissue, the pituitary gland. This can probably be accomplished by injecting the substances to be tested via the carotid arteries or by injection directly into the pituitary gland using stereotactic techniques.

The whole concept of releasing factors is so new that their potential uses in the control of animal reproduction are difficult to predict. If speculations are in order, one can anticipate that releasing factors may turn out to be a more natural means of controlling reproductive and other physiologic functions of animals than the injection of exogenous hypophysial hormones. This is predicted on the grounds that releasing factors act on the pituitary gland directly and govern the release of the animals' own hypophysial hormones. It remains to be seen whether some or all of the releasing factors also enhance the rate of synthesis of hypophysial hormones. Perhaps the use of releasing factors will permit one to get away from phenomena of under- or overstimulation, antibody formation, and other aberrations and obstacles to optimal results. Such difficulties are frequently encountered when exogenous hormones are injected.

On the basis of results obtained in rats and rabbits, it appears that it may become possible to control the time of ovulation by the use of LHRF. It remains unknown whether FSH- and LHRF's may provide a tool by which the number of maturing follicles may be increased and the rate of their maturation hastened. If these interventions turn out to be feasible, they may eventually provide a far more natural means of synchronizing estrus cycles than the ones presently used. Similarly, it appears possible that growth and perhaps fattening processes may be influenced by the use of TSHRF and somatotrophin RF. These hopes are predicated on the possibility of obtaining releasing factors in sufficient quantities for such applications. In view of the success of complete synthesis of such complex molecules as ACTH, synthesis of the relatively simple small RF molecules appears within reach. Perhaps the general interest of the scientific community and of commercial companies may hasten the day when synthetic RFs may become available in sufficient quantities for experimentation in domestic animals. In the meantime some groundwork can and should be laid by those of us interested in reproduction and in animal growth. Much valuable experience can be gained by experimenting on smaller animals such as guinea pigs and chickens on problems pertaining to reproduction and growth. The techniques of obtaining hypothalami and of extracting them for releasing factors are relatively simple and easy to master.

I would like to devote the remainder of my time to the discussion of phenomena which clearly involve participation of the hypothalamus in hypophysial control of reproductive function but which, in my opinion, have not been given the attention they deserve by American reproductive physiologists. I shall omit the details of the fascinating studies on rats and mice performed by Whitten and by Bruce which showed that odor of males is capable of profoundly modifying the reproductive behavior of females. The Whitten effect demonstrates that presence of males can in fact synchronize time of ovulation in a group of females by hastening or delaying heat and ovulation in a significant number of members of a mouse population. The Bruce effect is equally fascinating in that it shows that male odor can interrupt a previously initiated pregnancy at the early blastocyst stage by causing the cessation of release of hypophysial prolactin, the LTH of mice. (For details of the Whitten and Bruce effects, the interested reader should consult the papers by Bruce and by Parkes in "Advances in Neuroendocrinology.")

Reminiscent of the Whitten effect is the observation originally made in New Zealand and later confirmed in Tennessee that in sheep the onset of the breeding season may be advanced by weeks if ewes are exposed to rams several months prior to the expected onset of the breeding season as compared to ewes which had not been exposed (Smith et al., 5).

Some years ago we were greatly puzzled by the fact that pigs which did not settle to repeated services on their home farms and which were sold as being sterile to research workers at the University of Illinois, conceived at the first service after being shipped by truck to the new location. Our data showed that about 50 percent of the hogs sold as "sterile" had no anatomical or endocrine abnormalities (Wilson et al., 6). Infections, male sterility, or nutritional deficiences having been largely ruled out as the possible causes of "sterility," the truck ride was the only event that intervened between "sterility" and sudden fertility. The reason for this marked change in fertility level remains unknown. Similarly puzzling is the observation made by several independent workers but not adequately documented in the literature, that a significant amount of synchronization of sexual activity occurs in normal gilts if they are shipped from one place to another. About 5 to 7 days after arrival at the new place of habitation, a significantly greater number of shipped females show heat than would be expected to do so on a random basis. In one experiment (du Mesnil du Buisson et al., 1), 45 percent or 465 prepubertal females showed heat 1 to 9 days after being shipped, while only 180 females should have been in heat during this period had the random nature of this phenomenon not been disrupted by shipping. In addition to the effect of synchronization, the same experiment also indicates that the onset of sexual maturity was hastened either by the stress of transportation, or by the new environment, or by the daily checking of the females for heat by males.

The French group of workers has other studies which implicate the hypothalamo-hypophysial axis as a mediator in the modification of reproductive responses.

Domestic mammals show a variant of the long-known lordosis reflex of small laboratory mammals which has been analyzed in pigs by Signoret (4). He has confirmed the observations of many practical pig breeders that a sow in heat will become rigidly immobile when, in the absence of a boar, a man sits on her or even simply exerts manual pressure on the back. The degree to which this response can be obtained appears to be genetically controlled.

In cross-bred swine used for experimental work at the University of Illinois, 90 percent to 100 percent of all females respond to the test by rigid immobility. In the breed (Large White) used by Signoret, only about 50 percent of the females spontaneously responded to the signal, while the other 50 percent of the females in heat (and of course all females which are not in heat) escape attempts to be sat upon or attempts to exert manual pressure. Signoret noted that the proportion of females responding could be greatly increased if a male was present and he proceeded to fractionate the role of the male in eliciting the reflex. He found that 90 percent of the females showed the reflex if they could smell the males, (produced by infiltration of the area with male urine), and if they could listen to a recording of his typical rutting call. If, in addition to the odor and the call, they could also see the male, a further 7 percent showed the reflex, and if they could also have physical contact with him, the remaining 3 percent showed the reaction. The recorded call alone raised the response from 46 percent to 71 percent but, if the rhythm of the call was altered, only 9 additional percent of the females responded. Odor alone (impregnation of the area with the contents of the male preputial gland) raised the percentage of positive responses from 50 to 81 percent.

How the phenomena of sound and odor can intensify psychological heat, which is normally attributed to the action of estrogen on the central nervous system, remains completely unknown and invites further study. Of great interest is the fact that there is a very significant relationship between the readiness with which the rigidity reflex can be induced and the fertility of swine (table 1).

Olfaction in pigs, in addition to being related in some way to the intensification of the psychological heat response, is perhaps implicated in LH releases. Signoret and Mauleon (4) removed the olfactory bulbs from pigs and found that their cycles became irregular or ceased completely. The corpora lutea in the ovaries of these females disappeared, and there was an increase in the number of vesicular follicles which were about half as large as those of ovulatory size. These observations suggest the possibility that absence of olfactory bulbs does not interfere with FSH secretion but seems to prevent LH release either in toto or at least in sufficient quantities to cause the ovulatory spurt and ovulation. The morphology of these ovaries is reminiscent of the ovaries of rats in constant estrus which can be produced either by continuous lighting or hypothalamic lesions or by the single treatment of prepubertal rats with steroid hormones. In all of these cases the continuous follicular condition of the ovaries is known to be caused by a derangement of the LH storage and/or release mechanisms.

It was the intent of this brief review to call attention to some aspects of neuroendocrinology which remain, in my opinion, inadequately explored by those of us working with reproductive physiology of domestic animals. The involvement of the hypothalamus in the control of pituitary function can no longer be doubted. Some of the data reviewed strongly support the possibility that many environmental factors (odor, crowding, excitement, etc.), play an important role in significantly modifying the endocrine relationships between the hypothalamus-hypophysial axis and the endorgans. It seems important to start intensive investigation of the degree to which the exteroceptive factors do modify the endocrine stability of domestic animals, especially insofar as reproductive phenomena are concerned. It appears equally important to initiate research on the possible use of hypothalamic releasing factors in the control of physiological function of animals other than the laboratory rat.

Literature Cited

(1) du Mesnil du Buisson, F., and Signoret, J. P.
1962. Influence de facteurs externes sur le declenchement de la puberte chez la truie. Ann. Zootech. 11: 53.

(2) Nalbandov, A. V. (Ed.).
1963. Advances in Neuroendocrinology. Univ. Ill. Press, Urbana.

(3) Signoret, J. P., and du Mesnil du Buisson, F.
1962. Etude du comportement de la truie en oestrus. Economie et Medicine Animales, 3^e annee, p. 123.

(4) Signoret, J. P., and Mauleon, P.
1962. Action de l'ablation des bulbes olfactifs sur les mecanisms de la reproduction chez la truie. Ann. Biol. Anim. Bioch. Biophys. 2: 167.

(5) Smith, H. J., McLaren, J. B., Odom, J. A., and Miller, Howard.
1958. Influence of the Use of Sterile Teaser Rams Prior to Breeding on Subsequent Fertility of Ewes. Jour. Anim. Sci. 17: 1231. (Abst.)

(6) Wilson, R. F., Nalbandov, A. V., and Krider, J. L.
1949. A Study of Impaired Fertility in Female Swine. Jour. Anim. Sci. 8: 558

Table 1.--Relation of conception rate in swine to their spontaneous ability to show the "immobility reflex"

	Nullipara		Multipara		All Females	
Behavior	Total number	Percent conceived	Total number	Percent conceived	Total number	Percent conceived
Very calm	351	56.7	520	68.6	871	63.8
Calm	1120	49.4	1507	54.5	2627	52.3
Agitated	345	31.3	461	36.2	806	34.1
No information	16	68.7	18	55.5	34	61.7
All	1832	47.5	2506	54.1	4338	51.3

Data adapted from: du Mesnil Du Buisson. 1961.
Ann. Zootech. 10:57-67.

THE ROLE OF LIGHT IN THE REGULATION OF CYCLICAL ESTROUS ACTIVITY IN SHEEP[1]

M. T. Clegg, H. H. Cole, and W. F. Ganong
Department of Animal Husbandry, University of California,
Davis, and Department of Physiology, University of California,
School of Medicine, San Francisco, California

The widespread existence of biological clocks among animals is documented by a considerable body of evidence. Recently Farner (13) has reviewed this subject covering information on a large variety of animals. In this paper an attempt will be made to restrict most of the discussion to sheep. Experimental data from other species, however, will be cited when it can be usefully related to an understanding of the mechanisms controlling seasonal breeding activity in this species. As far as present knowledge is concerned, the factors regulating or at least monitoring annual physiologic cycles appear to depend greatly upon the influence of changes in the environment and experimental evidence supports the idea that the amount of daily light to which the animals are exposed is responsible for the initiation of the breeding season (Sykes and Cole, 23; Hafez, 16; Hart, 18; Yeates, 29).

This concept, however, does not account for the considerable variations in seasonal breeding activity of different breeds of sheep; nor does it explain the difficulty in producing prolonged anestrus with any photoperiodic treatment.

It is the purpose of this paper to review briefly the evidence for photoperiodic control of estrus in sheep together with a report of our own studies on the influence of controlled lighting, optic nerve section, and destruction of the posterior optic chiasm on this phenomenon. Finally we should like to reserve the right to speculate by presenting alternate explanations involving light receptors other than the retina and to demote the influence of light to that of a modifier of an inherent biological rhythm.

Considerable data concerning the influence of environmental factors on the ewe have been forthcoming since the classical paper of Marshall (22). It was his thesis that the development of photoperiodism could be explained by natural selection. Thus only those animals in a harsh environment who developed the ability to time the birth of their young during that part of the year when survival was possible would persist. He suggested that incidence of daylight was an important factor in controlling the cycle and that the sheep reacted to a diminution rather than an increase in length of daylight. These ideas were supported by a number of observations. In many breeds of sheep, in the northern hemisphere, breeding activity begins about August or September (a time of decreasing hours of light) and lasts until late February or early March when daylight is increasing. In sheep transported from one hemisphere to another, breeding season tends to be reversed (Marshall, 22).

Direct experimentation has confirmed that diurnal light-dark rhythm plays an important role affecting reproductive photoperiodicity. Sykes and Cole (23) were the first to demonstrate that if light was gradually decreased, beginning in March and extending through May, ewes could be bred in May and June, a time of normal anestrus. Others utilizing artificial illumination to reverse the seasonal changes in length of daylight have confirmed these findings (Yeates, 29; Hafez, 16). Much evidence, however, does not support a shortening day length as the appropriate stimulus for the initation of a reproductive rhythm. For example, some breeds of sheep, such as the Corriedale and Dorset Horn, may exhibit estrus during the spring and summer months and others, such as the Merino, may breed at almost any season of the year, but, even in the latter, periods of reproductive activity alternate with periods of quiescence. Further, the light to dark ratio does not appear to be a critical factor in the induction of estrus. Hart (18) maintained sheep on a constant light rhythm: either 4 L and 2 D, then 4 L and 14 D; or 4 L and 8 D, then 4 L and 8 D. Either treatment was successful in producing a regular series of estrous cycles; Tsimborovich (27) obtained similar results with 11 D, 3 L, 3 D, 7 L and 11 D, 3 L, 2.5 D, 7.5 L; Hafez (16) found that a fixed ratio of light to dark (8 L, 16 D) was sufficient to bring about cyclical reproductive patterns and Terry and Meites (24) showed that sheep kept for seven weeks in summer on 24 hours of light, no light, or on daylight all continued to cycle.

Since most of these experiments were designed to test different types of stimuli, either length of the dark period or light to dark ratios, on the induction of estrus, the question remains as to whether patterns of cyclical reproductive activity occur independent of season when a constant light regime is imposed. An answer would provide presumptive evidence for the presence of an

---

[1] These studies supported in part by USPHS Grant B3977.

inherent biological rhythm capable of being modified by diurnal changes in light-dark rhythm but not exclusively dependent upon them. An answer to this question is provided by an experiment carried out several years ago by Dr. H. H. Cole, Dr. William C. Weir, and the author at California.

Effect of a constant light regime

Two experimental groups consisting of four Suffolk and five Hampshire ewes each were constituted. The ewes ranged in age from two to six years. The treated group was placed in rooms constructed to eliminate all light from the outside. These rooms were equipped with electric clocks which automatically turned two 150 W lights on at 9:00 a.m. and off at 3:00 p.m. each 24-hour period, thus exposing the ewes to six hours of artificial light daily. Light intensity ranged from 2 to 5 f.c. depending upon the distance from the light source. The sheep were placed under this treatment regime in August and were kept continuously under these conditions for a period of three years ending in May.

The control ewes were kept in outside pens adjacent to the "blacked-out" rooms and subjected to normal environmental light changes.

All animals were checked daily for receptivity to the male by placing them with vasectomized rams. Period of checking was always during the time of light exposure. Both groups received the same ration which consisted of alfalfa hay and occasional supplementation with barley.

Figure 1 summarizes and compares the normal seasonal periods of reproductive activity and quiescence observed over a two or three year duration for three breeds of sheep, Suffolk, Hampshire, and Suffolk-Hampshire crosses under central California conditions. The percentage of estrus in this figure, as well as figures 2, 3, and 4, is based upon the assumption that each ewe should show approximately two cycles per month. Therefore, 100 percent would be equal to twice the total number of animals. By using this method, additional information concerning the irregularities of estrous cycles can be shown.

To be noted is the uniformity of responses from year to year. Thus the period of anestrus predominates from April to July and peak activity generally occurs from October through January.

No differences in reproductive activity between the Hampshire and Suffolk ewes were observed.

Figure 2 depicts the effect of a constant light regime on seasonal estrous patterns. After the first year of treatment there is a tendency for the periods of activity to become prolonged and the length of the period of anestrus to be greatly shortened. The number of occurrences of heat during April, May, June, and July in the treated group was increased whereas, during these same months, in the control group, heat is largely absent. Cycles become progressively less regular under the constant light regime. Rarely do the number of observed heats exceed 50 percent. In contrast, the number of control animals showing estrus during the late fall and winter months ranged from 60 percent to almost 100 percent after the first year of the experiment. Nevertheless, a cyclical pattern of increasing and decreasing reproductive activity is still apparent in spite of the prolonged period of treatment with a constant light-dark duration. The premise that the progressive diminution in number of daylight hours acts as the stimulus for the induction of estrus is not supported by the results of this study. Rather the implication seems clear that changes in diurnal light-dark ratios appear to be capable of modifying or monitoring an inherent biological rhythm.

Light receptors and neural pathways

How this effect of light influences the endocrine system and ultimately gonadal activity has yet to be explained. As early as 1936 Marshall stated ". . . . the reproductive rhythm, . . ., is, in higher forms of life, regulated further by exteroceptive stimuli conveyed by nervous paths to the hypothalamus and thence transmitted to the anterior pituitary." This concept still prevails, although there is little direct proof of the neural pathways involved and, with few exceptions, only circumstantial evidence supports an endocrine effect.

The obvious receptor for light stimuli associated with reproductive photoperiodism is the eye. Bissonnette (3) found that the female ferret blinded by a section of the optic nerves either remains anestrus or comes into estrus at irregular intervals totally unrelated to the normal pattern. Some difficulties in the interpretation of experiments such as this occur because estrus will develop independently of photoperiodic stimulation. Thomson (26) has shown that blinded ferrets come into estrus at the normal time and suggests that photoperiodicity is not involved in the normal timing of the estrous cycle. Nevertheless, Hammond (17) has summarized considerable evidence that, at least in the ferret, the receptors are retinal since sectioning of the optic nerve eliminates the photoperiodic effect.

If the eye is the receptor in mammals, then the nerve pathways must involve the optic nerve and other fiber tracts impinging upon hypothalamic nuclei which regulate pituitary gonadotrophin secretion by way of releasing factors. The neural pathways by which extra light gain access to the hypothalamopituitary axis are largely unknown. Donovan and Harris (11) have shown that pituitary stalk section blocks the response to artificial illumination in the ferret and hypothalamic lesions accelerate the onset of light-induced estrus in this animal (Donovan and Vander Werff ten Bosch, 12). Clark and associates (6) studied the effect of lesions of the visual system on the response to artificial illumination in ferrets. They found that although optic nerve section blocked the response, removal of the entire visual area of the occipital cortex, destruction of the superior colliculi, or the ablation of all the lateral geniculate body except its ventral nucleus did not interfere with the normal response to illumination. From this experiment they concluded that impulses originating in the retina were transmitted to the hypothalamus by way of the ventral nuclei of the lateral geniculate body and accessory optic tracts. In a subsequent study, however, no evidence of such connections were found (Jefferson, 19). More recently, Critchlow and de Groot (10) showed that, in rats, made constant estrus by constant lighting, optic enucleation or lesions in the suprachiasmatic region abolished this effect; whereas lesions of the optic tracts were ineffective. From these results they concluded that the classical and accessory pathways posterior to the chiasm were not involved in the transmission of light sensory modalities, but postulated the presence of retino-hypothalamic connections which pass through the chiasm and preoptic region to structures controlling gonadotrophin secretion.

It should be emphasized that most experiments of the types just described have involved only two species, the rat and the ferret. As far as this author knows, comparable experiments have not been performed in sheep.

Effect of blinding and chiasmatic lesions

Recently, in collaboration with Dr. W. F. Ganong, a series of experiments were designed to determine whether sectioning of the optic nerve or destruction of the posterior portion of the optic chiasm together with the suprachiasmatic region would affect sexual periodicity in this species.

For the first trial nine Suffolk-Hampshire crossbred, 2-year-old ewes were selected from the University breeding herd. Daily observations for estrous activity were begun on the first of June by determining receptivity to either vasectomized or aproned rams and continued until the end of the experiment in March, 22 months later.

On June 15 the optic nerves were severed by surgical procedures in four ewes randomly selected from the nine. The remaining five served as intact controls. Ewes of both groups were placed together in a large pen, fed the same ration of chopped alfalfa hay, and exposed to the same environmental influences.

In the second trial ten 8-year-old Corriedale, cross-bred ewes were divided and treated as follows:

In three animals destructive lesions were placed in the region of the posterior optic chiasm and suprachiasmatic area according to techniques previously described (Clegg et al., 8). Two animals served as operative controls. In these ewes, lesions were placed in areas other than described above but care was taken to avoid the anterior median eminence, an area in which previous studies had shown to cause an effect on reproductive activity (Clegg and Ganong, 7). The remaining five ewes served as intact controls. All operative procedures were carried out between June 10 and June 15.

As in the previous trial all animals were placed together in the same pen and exposed to the same nutritional and environmental conditions.

Beginning on the first of June, daily checking for estrous activity based on receptivity to vasectomized rams was initiated and continued until the end of the trial, 22 months later. At the end of the trial, the animals were sacrificed. The hypothalami of the operated animals were removed, fixed, serial sectioned, and stained. From studies of this material, it was possible to reconstruct the area of the lesion.

Effects of blinding

Figure 3 illustrates the influence of optic nerve section on reproductive activity. The lack of effect of blinding upon seasonal estrous activity is the remarkable finding. It is true that more irregular cycles occurred in the blind sheep than in the controls, but since these blind animals were in a large pen some difficulty may have been encountered on the part of the rams in seeking out these ewes. Some evidence exists that the estrous ewe actively approaches the ram (Lindsay and Robinson, 20). The fact that estrous activity was observed in April, normally a time when no cycles are observed, could suggest some effect of blinding, but the significance of this observation must await further studies. To account for the lack of a dramatic effect of optic nerve section, two interpretations are offered.

(1) The stimulus required to induce estrous activity must depend upon a change in the light, dark duration. If the receptor for light is destroyed, the animal reverts to an inherent biological rhythm which, in the case of the sheep, coincides with the seasonal variations in reproductive activity.

(2) Light receptors other than the eye exist, possibly in the brain, which respond to seasonal changes in the duration of daylight.

Let us examine these two possibilities in the light of current experimental data.

Whereas light appears to be stimulatory to processes regulating gonadal function, the effects of exposure to constant dark or blinding are less dramatic. Thomson (25) observed estrus in blind ferrets during the normal time of year and Browman (4, 5) saw no effects in rats. These results together with the findings in sheep clearly indicate that a photoperiodic mechanism is not essential for the development of estrus. Rather, light appears to contribute to the regulation of a seasonal pattern associated with some inherent biological rhythm. Critchlow (9) prefers an alternate view. He suggests that rhythmic changes in other environmental factors, such as temperature and humidity, take over a stimulatory function in the absence of light.

The existence of light receptors in the brain has been suggested by the experiments of Benoit and Assenmacher (2). After removal of the eyes, in male ducks, light focused on the pituitary, hypothalamus, or rhinencephalon was effective in evoking gonadal stimulation. In case of the larger mammals, particularly the sheep, it is difficult to visualize that sufficient light could be capable of being transmitted through the wool and thick skull to the deep areas of the brain. Recently, Ganong et al. (15) reported on the results of an experiment bearing on this problem. They found, following the insertion of a photovoltaic cell into the hypothalamus and temporal lobe of sheep, dogs, rabbits, and rats, that, indeed, light was able to penetrate the brain. They emphasize, however, that no physiological function of light on brain receptors was implied. It is an intriguing thought to consider that light receptors do exist within the brain of sheep.

Effects of chiasmatic lesions

Figure 4 shows the effect of destruction of the posterior optic chiasm and suprachiasmatic area on sexual photoperiodicity. The data suggest that this procedure does, indeed, influence the normal seasonal response, but a prolonged post-operative period is required before a demonstratable effect can be observed. The principal influence of treatment is to bring about periods of anestrus during that part of the year when gonadal activity is normally greatest. That this effect is confined to the optic chiasma and suprachiasmatic area and is specific is indicated by a lack of influence of destructive lesions in other areas of the hypothalamus, excluding the anterior median eminence.

Had blinding produced a similar effect one might conclude that the optic nerve and retinohypothalamic tracts represented the important neural pathway by which light stimuli influence the photoperiodic response. Since this was not the case, other explanations must be sought.

The observation that cervical sympathectomy delays the onset of photoperiodically induced estrus in ferrets (Abrams et al., 1) implies an influence of the sympathetic nervous system. In a very recent report by Wurtman et al. (28), bilateral extirpation of either the eyes or the superior cervical gauglion blocked the stimulating effect of light which has been shown to cause an increase in the incidence of estrus in rats and to depress melatonin synthesis. From these results, they suggested that one pathway whereby light influences the rat estrous cycles involves the retina, sympathetic nervous system, and the synthesis of melatonin by the pineal. An involvement of the sympathetic system in controlling the photoperiodic response in sheep cannot be discounted. It is known that the hypothalamus plays an important role in thermoregulatory functions and there is evidence, in the rat, that anterior hypothalamic damage in the preoptic region is associated with changes in autonomic nervous activity (Gamble and Patton, 14; Maire and Patton, 21). In those sheep sustaining lesions in the suprachiasmatic area damage to sympathetic fibers need not be unexpected and could explain the decreased incidence of estrus observed in this experiment.

Summary

Evidence provided by these experiments as well as others clearly implicate the role of light in modifying sexual periodicity in sheep. The neural pathways by which light stimuli influence the hypothalamo-hypophyseal axis are not clearly understood. In sheep, sectioning of the optic nerve does not cause a dramatic effect. The absence of an influence suggests that rhythmic changes in light duration is the stimulus and acts to precisely regulate the frequency and phases of an inherent biological pattern. That other light receptors, however, may be present in the brain cannot be ruled out. Finally, pathways other than the optic nerve and visual tracts may also conduct environmental stimuli capable of influencing hypothalamic nuclei and ultimately gonadal activity. Thus lesions in the optic chiasm reduce the incidence of estrus and may involve destruction of sympathetic pathways.

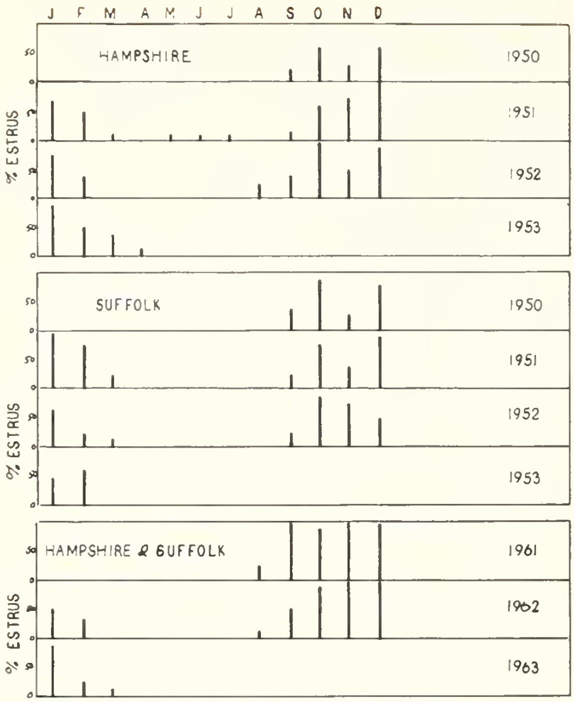

FIGURE 1. A comparison of seasonal estrous activity for different years in several breeds of sheep. Note the uniformity of months of activity and of anestrus.

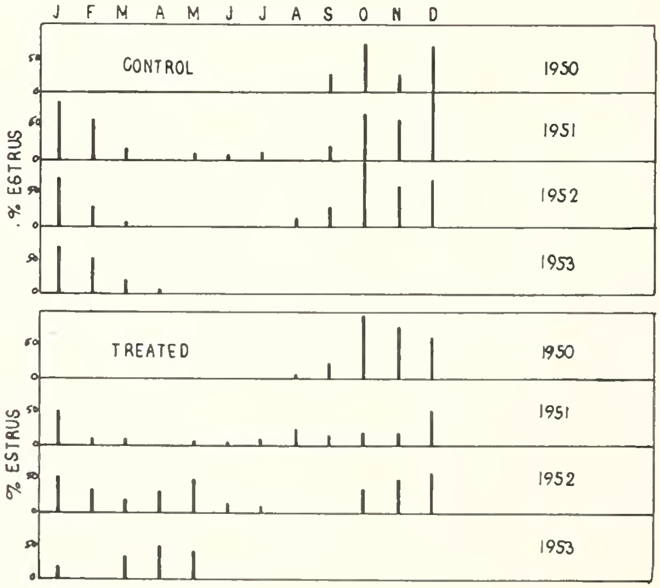

FIGURE 2. The effect of a constant light regime (6 hrs. light, 18 hrs. dark) on seasonal estrus in Suffolk and Hampshire sheep. Note the alteration in seasonal patterns of activity.

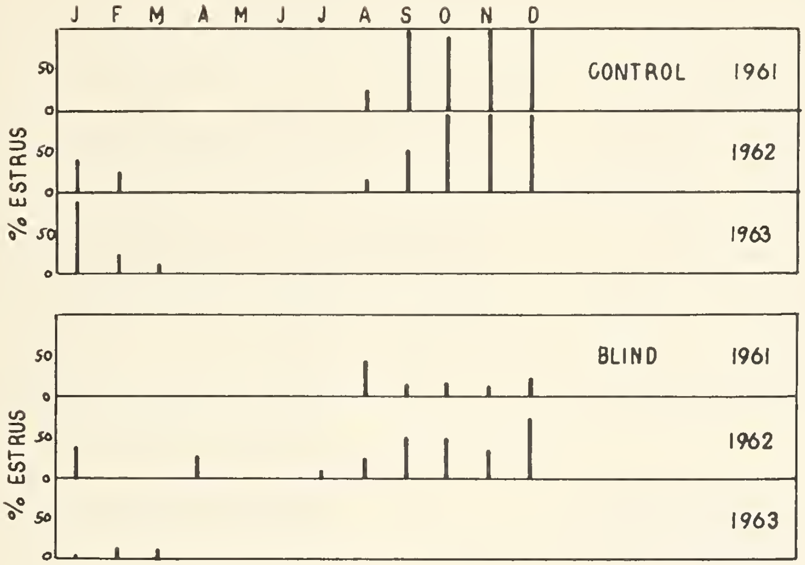

FIGURE 3. The effect of sectioning of the optic nerve on the percentage of estrus during different months and years in crossbred Suffolk-Hampshire sheep. To be noted is the lack of effect of blinding on seasonal estrus.

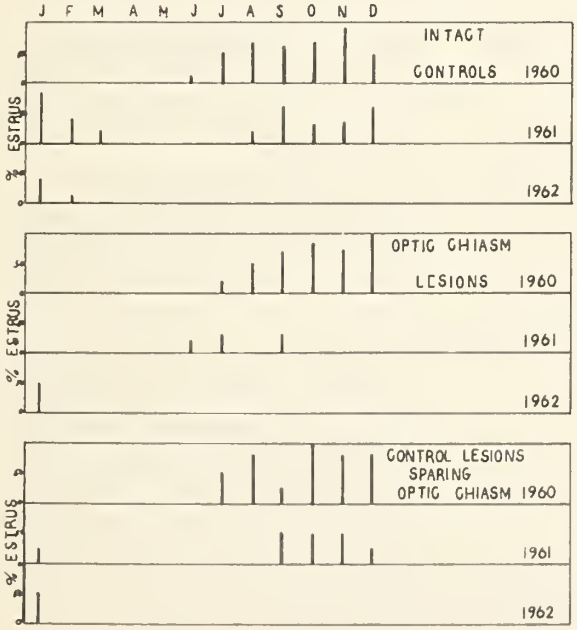

FIGURE 4. The effect of chiasmatic lesions on the percentage of estrus in crossbred Corriedale sheep. Lesions in this area tend to reduce incidence of estrus.

Literature Cited

(1) Abrams, M. E., Marshall, W. A., and Thomson, A. P. D.
1954. Effect of Cervical Sympathectomy on the Onset of Oestrus in Ferrets. Nature 174: 311.

(2) Benoit, J., and Assenmacher, I.
1959. The Control by Visible Radiations of the Gonadotropic Activity of the Duck Hypophysis. Recent Progress in Hormone Research 15: 143. Academic Press, New York.

(3) Bissonnette, T. H.
1938. The Influence of Light upon Pituitary Activity. Proc. Assn. Res. Nerv. Ment. Dis. 17: 361.

(4) Browman, L. G.
1937. Light in its Relation to Activity and Estrous Rhythms in the Albino Rat. Jour. Expt. Zool. 75: 375.

(5) Browman, L. G.
1943. The Effect of Controlled Temperatures upon the Spontaneous Activity Rhythms of the Albino Rat. Jour. Expt. Zool. 94: 477.

(6) Clark, W., Le Gros, E., McKeown, T., and Zuckerman, S.
1939. Visual Pathways Concerned in Gonadal Stimulation in Ferrets. Proc. Roy. Soc. London Ser. B. 126: 449.

(7) Clegg, M. T., and Ganong, W. F.
1960. The Effect of Hypothalamic Lesions on Ovarian Function in the Ewe. Endocrinology 67: 179.

(8) Clegg, M. T., Santolucito, J. A., Smith, J. D., and Ganong, W. F.
1958. The Effect of Hypothalamic Lesions on Sexual Behavior and Estrous Cycles in the Ewe. Endocrinology 62: 790.

(9) Critchlow, V. J.
1963. The Role of Light in the Neuroendocrine System. Advances in Neuroendocrinology. Ed. A. V. Nalbandov. Univ. Ill. Press, Urbana.

(10) Critchlow, V. J., and de Groot, J.
1960. Experimental Investigations of Pathways Involved in Light-Induced Constant Estrus in the Rat. Anat. Rec. 136: 179.

(11) Donovan, B. T., and Harris, G. W.
1954. Effect of Pituitary Stalk Section on Light Induced Oestrus in the Ferret. Jour. Physiol. (London) 131: 102.

(12) Donovan, B. T., and van der Werff ten Bosch, J. J.
1959. The Relationship of the Hypothalamus Oestrus in the Ferret. Jour. Physiol. (London) 147: 93.

(13) Farner, D. S.
1961. Comparative Physiology: Photoperiodicity. Ann. Rev. Physiol. 23: 71.

(14) Gamble, J. E., and Patton, H. D.
1953. Pulmonary Edema and Hemorrhage from Preoptic Lesions in Rats. Amer. Jour. Physiol. 172: 623.

(15) Ganong, W.F., Shepherd, M. D., Wall, J. R., Van Brunt, E. E., and Clegg, M. T.
1963. Penetration of Light into the Brain of Mammals. Endocrinology 72: 962.

(16) Hafez, E. S. E.
1952. Studies on the Breeding Season and Reproduction of the Ewe. Jour. Agr. Sci. 42: 13.

(17) Hammond, J., Jr.
1954. Light Regulations of Hormone Secretion. Vitamins and Hormones 12: 157.

(18, Hart, D. S.
1950. Photoperiodicity in Suffolk Sheep. Jour. Agr. Sci. 40: 143.

(19) Jefferson, J. M.
1940. A Study of the Subcortical Connexions of the Optic Tract System of the Ferret, with Special Reference to Gonadal Activation by Retinal Stimulation. Jour. Anat. 75: 106.

(20) Lindsay, D. R., and Robinson, T. J.
1961. Studies on the Efficiency of Mating in Sheep. II. Effect of Freedom of Rams, Paddock Size and Age of Ewes. Jour. Agr. Sci. 57: 141.

(21) Maire, F. W., and Patton, H. D.
1954. Hyperactivity and Pulmonary Edema from Rostrate Hypothalamic Lesions in Rats. Amer. Jour. Physiol. 178: 315.

(22) Marshall, F. H. A.
1937. On the Change Over in the Oestrous Cycle in Animals after Transference across the Equator, with Further Observations on the Incidence of the Breeding Seasons and the Factors Controlling Sexual Periodicity. Proc. Roy. Soc. London Ser. B. No. 829, Vol. 122: 413.

(23) Sykes, J. F., and Cole, C. L.
1944. Modification of Mating Season in Sheep by Light Treatment.

Mich. State Univ. Agr. Expt. Sta. Quart. Bul. 26: 250.

(24) Terry, W. A., and Meites, J.
1951. The Effects of Continuous Light or Darkness on Thyroid and Reproductive Function in Ewes. Jour. Anim. Sci. 10: 1081.

(25) Thomson, A. P. D.
1951. Relation of Retinal Stimulation to Oestrus in the Ferret. Jour. Physiol. (London) 113: 425.

(26) Thomson, A. P. D.
1954. The Onset of Oestrus in Normal and Blinded Ferrets. Proc. Roy. Soc. (London) B142: 126.

(27) Tsimborovich, A. F. 1955. Cited by A. Emme.
1958. Svyet i Zhizn (Gosydarstvyennoye Izdatyelyestvo Syelsko-Khozyaistvyennoi Lit., Moskov, U.S.S.R.

(28) Wurtman, R. J., Axelrod, J., Chu, E., and Fischer, J. E.
1964. Mediation of Light-Induced Persistent Estrus by the Rat Sympathetic Nervous System. Sixth Ann. Meeting of the Endocrine Soc., San Francisco, p. 23.

(29) Yeates, N. T. M.
1949. The Breeding Season of the Sheep with Particular Reference to its Modification by Artificial Means Using Light. Jour. Agr. Sci. 39: 1.

DR. NALBANDOV: Thank you, Dr. Clegg, for a very illuminating talk.

DR. ZIMBELMAN: Has the type of animal been studied in terms of the source of the hypothalmic releasing factor? In other words, if you are using sheep, is it important whether they are wethers or ewes?

DR. NALBANDOV: As far as we know, the reproductive condition of the donor has no major effect on the RFs. The hypothalami have been collected indiscriminately from all slaughtered animals that happened to go through and, so far, the efforts have been directed simply towards the characterization of the releasing factors, without regard to the actual sex differences that may exist.

DR. MELAMPY: Is there a relationship between the uterus and releasing factors? I am thinking of hysterectomized, unmated females or pregnant females.

DR. NALBANDOV: As you know, there is no experimental evidence. My guess is that the relationship is very profound between the uterus and the hypothalmic centers, on the basis of Hansel's work and on the basis of the work that you have done yourself and the work we have done on the beading, the transplantation of live embryos that is now being conducted in Cambridge. All indications are that there is a profound relationship between the uterus and the pituitary gland. The only way in which information from the uterus can be transmitted to the pituitary appears to be through the hypothalamus.

DR. HANSEL: Which of the current methods for measuring LHRF do you favor; the Nikitovitch-Winer type where you instill the fraction directly into the pituitary in an attempt to induce ovulation; or the McCann type where you attempt to measure LH in the blood after giving the fraction?

DR. NALBANDOV: We have not done any measurements of LHRF. We have backed off from this as much as possible, although it may be that we are going to be forced into this in order to characterize some of the peculiarities that we are finding in our own work with hypophysectomized males. My plan is to use the McCann method which utilizes the Parlow test for the assay of LH release.

I would like to ask Dr. Parlow what information he has on the effect of hypothalamic releasing factors on the ascorbic acid depletion of the ovary of rats prepared according to his specifications for LH assay.

DR. PARLOW: In early work done by Dr. McCann the impression was gained that actually only a minimal amount of ovarian ascorbic acid depletion could be attained with LHRF. However, it is now clear--from more recent work done by Schally and Bowers--I have not done any of this myself--that with a sufficiently highly purified preparation of LHRF so that you can administer an adequate dosage to the test animal, very substantial depletion of ovarian ascorbic acid is possible.

DR. NALBANDOV: I missed this work. Has it been published?

DR. PARLOW: Yes. Endocrinology 75: 312, 1964.

DR. HANSEL: Does vasopressin give a similar depletion?

DR. PARLOW: In sufficiently high dosage, Dr. Hansel, it will. But this is a very different kind of phenomenon. The dosage of vasopressin required to give such a response is so large that during the process of intravenous injection we nearly kill the animal. This is a very different kind of effect; an effect directly on the ovary.

DR. NALBANDOV: How much depletion did we get with synthetic vasotocin?

DR. GIBSON: It was rather small, I think. We never did run very many animals. It was something on the order of 10 percent, I think.

DR. BELLOWS: Would you care to speculate on what you are picking up in the blood on the hypophysectomized cock that you mentioned?

DR. NALBANDOV: Yes. Speculations only. The peculiar thing is that as it stands now there is nothing in the blood of the hypophysectomized cock during the first three weeks after hypophysectomy. The blood is completely negative in the Parlow assay.

After three weeks a substance appears which gives maximal depletion. We get depletions of 50 to 60 percent, and this substance persists in the blood of these hypophysectomized cocks.

Now the cocks themselves do not respond to whatever this substance is by any demonstrable testicular or comb growth. To all intents and purposes they are hypophysectomized and a check of the sella turcica shows that they are cleanly hypophysectomized. The question then arises: What is it? This is what we are in the process of trying to find out.

The reason we tried vasotocin was because one of the suspicions was that it may be a neuro-hypophysial factor that gives the depletion. However, even with large dosages of vasotocin we did not get the maximal depletion that we get with the substance in the blood.

We are in the process of trying to see whether it is a releasing factor or a peculiar kind of LH, an LH precursor, or what. This is the aim of our present efforts of clarifying the situation.

DR. FAULKNER: Are you able to remove the tuberalis in hypophysectomy in the cock?

DR. NALBANDOV: Little pieces of tuberalis persist. They cannot be removed completely. But my guess is it is not the source of whatever this substance is, for the simple reason that these bits of tuberalis are present immediately after the hypophysectomy and during the first three weeks after hypophysectomy when this blood factor is absent.

DR. FAULKNER: In the hypophysectomized rat occasionally we see castration-like cells develop in the tuberalis.

DR. COLE: I would like to raise a point in the discussion of an inherent rhythm. The question occurs to me now, belatedly, why doesn't the ewe take up the inherent rhythm of Australia rather than California? In other words, why do they necessarily come in in October, when we know that in the Southern Hemisphere they come in in another season of the year? This raises the question in my mind of the extent to which other environmental factors may possibly be influencing this result. Perhaps your cervical sympathetic studies might give a clue to this. And also we know of the work of Professor Dutt showing that temperature might also be involved.

I wonder if Dr. Clegg would want to comment on this?

DR. CLEGG: Your point is very well taken. I don't think we can deny that light has an influence, as well as other environmental stimuli in normal patterns of cyclical behaviour or cyclical reproductive activity. But I think it is important to realize that light is acting only as a modifier.

We can only speculate on how this works. Presumably light affects thresholds of activity of some central nervous neurons. But this is superimposed upon a normal rhythm which exists without light.

I don't know whether that gets by your point or not, but I don't think that one can deny the importance of light or at least other environmental stimuli.

DR. COLE: I am wondering if possibly starting with pre-pubertal animals or before sexual maturity, might be of importance here. In other words, the inherent rhythm we speak of may be related to the time you place animals on a light regime.

DR. CLEGG: But you answered that question by simply saying that in changing from one hemisphere to the other they revert to the seasons to which they are transplanted. Thus this cannot be a sort of a conditioned response.

Certainly the influences of steroids on pre-pubertal or post-pubertal heat does have a striking influence upon subsequent hypothalamic regulation of reproductive activity. And these are the classic studies of the testosterone injections or the castration of the 4-day-old rat, but I am not sure that light would produce such an effect.

DR. NALBANDOV: Perhaps light works via the releasing factors. Dr. Lloyd Anderson has just returned from Jouy-en-Josas a short time ago and I would like to ask him a question.

Du Mesnil du Buisson, at the time I was there a year ago, was trying to see whether ovaries with persistent follicles in the anosmic pig can be made to ovulate with LH. Has he done this experiment yet?

DR. ANDERSON: No, he hasn't.

DR. CASIDA: Do we have something of a direct relationship between dosage of progestogen that has been administered in attempting to synchronize estrus and the interval of time from withdrawal until the animal comes into estrus? I believe you have made quite a point in your own work of the long interval of a very minor follicular development after mammoth doses of MAP. At what level do you speculate that this is working? Why this correlation between dose and latent interval?

DR. NALBANDOV: I really don't know. What goes through my mind right now is this pre-pubertal steroid castration effect which is clearly not a dose effect, but manifests itself long after steroid injection.

By the way, I also might mention, just to clear up a few points, that more recent work has shown that this pre-pubertal castration effect is not an all or none effect that we had been led to believe by earlier work. Many of these rats do cycle, although the cycles are irregular, so that this is not an irreparable damage to the hypothalamus. What I was leading up to, then, is that perhaps the effect of steroids on the hypothalamic centers is simply a question of the rate at which the centers can recover from the damage that is inflicted on them. Nobody knows what "recovery" of the hypothalamus means in physiological terms.

At one time we became interested in hypothalamic lesions in sheep and inflicted a great many lesions in a great many sheep and essentially agreed with the work of Clegg and Ganong in that these hypothalamic areas that are damaged do have a definite influence upon the estrous cycle of sheep. The reason we went into this was because we had, and still have, a suspicion that in many instances these lesions are damaging the stalk area, therefore interrupting the hypothalamo-hypophyseal connection. We were very careful to keep away from the stalk area and put the lesions in other regions of the median eminence.

Now the interesting thing is that the immediate effect was that we would interrupt the estrous cycles, either heats would be absent or ovulations would be absent, or both would be absent. However, in practically all cases, if the sheep were kept for one or two breeding seasons, they recovered completely from these lesions and began to cycle perfectly normally. The still more puzzling thing was that the recovery was only physiological, so to speak, because on sectioning the brains we found the anatomic damage persisting even into the second and third seasons. The burned-out areas were there. Nevertheless the hypothalamus developed some mechanism by which they would bypass these lesioned, damaged areas, and revert to perfectly normal function.

This, by the way, is apparently true of other animals as well and is not restricted to sheep. Somebody has kept guinea pigs for prolonged periods of time after lesioning and found that they recover completely.

DR. CASIDA: Am I to infer that you believe that progestogen is working then at the hypothalamic level, that is resulting in some sort of paralysis of function there from which it recovers slowly, using paralysis in a very broad and general sense?

DR. NALBANDOV: This would be my guess. But I underscore the word guess.

DR. CLEGG: In the study I reported I omitted some of the data in which we had median eminence lesions purposely because these we knew affected reproductive activities. In our work with median eminence lesions we find that one out of three sheep may recover.

In respect to Dr. Casida's remark, we have just completed some work on the effects of progesterone with respect to gonadotropin release. These studies were done on wethers, purposely selected because we could detect gonadotrophic activity in the plasma of the cavernous sinus. If we injected progesterone over a period of 10 days, at the rate of 20 mg. daily, we find that during the period of injection there is no apparent effect on the gonadotrophic concentration in the blood. Rather we find a release or at least a very significant elevation of concentration which occurs at a very uniform time period, about the fifth or sixth day of treatment. And similarly, after withdrawal of the treatment, we again find this significant increase in concentration five or six days later. What the uniformity of this kind of response relationship to both progesterone treatment and withdrawal means, we do not know. We would like to guess that perhaps this stimulus is acting at hypothalamic level and that the stimulus depends upon a change in concentration; whether it is ascending or descending is unimportant.

DR. NALBANDOV: Thank you, Dr. Clegg. Dr. Hansel, would you like to contribute to this discussion that we have just heard? I think you are in a position to do so.

DR. HANSEL: I don't know whether there is really very much that I can add except that you noted in the data that Dr. Donaldsen presented yesterday on chronic oxytocin treated cows, we see the same thing Dr. Clegg has just mentioned. Initially a release, a depletion of pituitary gonadotropin and, despite continued oxytocin, there is a reaccumulation in the pituitary four days later. The corpus luteum is not affected. If anything, it is a little bigger and has a little more progesterone in it at this time. But by seven days the gonadotropin content of the pituitary has gone down again and the corpus luteum is markedly depleted of progesterone and its weight is down. In other words, we are seeing this same phenonenon it seems.

DR. CLEGG: I should like to make a plea that we utilize our plasma technique. We utilize the technique of collecting plasma from the pituitary. I must confess that I am not completely sold upon pituitary content.

DR. HANSEL: May I make a further plea? We need your help. I think all of us need your help. We have been able to collect cavernous sinus plasma from the cow as you have from the sheep. But to be perfectly honest, we have not been able to do anything with it. We cannot make any of the LH assays that have been published really work to our satisfaction. We have tried. We tried Zn^{65} uptake. We are all agreed that Dr. Parlow's assay is the best available. But these other things that have the sensitivity that are required simply do not work in our hands. We tried the cholesterol depletion of Loraine and Bell; it did not work. We tried Zn^{65} uptake in the testes of the hypophysectomized male rat and we did not get results with this. We need some help.

DR. NALBANDOV: In our hands the ascorbic acid depletion test on chicken plasma works extremely well. Now, Dr. Parlow, would you like to make some comment?

DR. PARLOW: I have nothing to suggest, except you might concentrate the plasma by extraction and then hope to do tests for activity by the OAAD method. There is no doubt about the fact that although the OAAD method is highly workable with proper attention to technical detail, it lacks the sensitivity required to measure LH activity in normal blood, with the same precise quantitation one can do this for LH activity in the pituitary.

DR. CLEGG: I should like to highly recommend Dr. Parlow's assay for LH. We find levels in ewe plasma of about .3 of a gamma compared to NIH standard S7 and S2, I don't know, but it is one of the two. And, again, with respect to Dr. Parlow, we have examined levels of both FSH and LH in the plasma of ovariectomized castrate sheep comparing them with intact sheep. Although we can find around .3 to .4 of a gamma per milliliter or microgram per mill, in the intact animals, LH activity does not, as far as we can tell, increase following castration. With the Steelman-Pohley assay for FSH, we have never been able to detect levels in intact animals either rams or ewes. However, we can detect up to about one-half microgram per mill in the ovariectomized or orchidectomized animal.

DR. MELAMPY: I would like to ask Dr. Clegg, in the hypophysectomized female rat, have you observed antrum formation with the plasma extract?

DR. CLEGG: Yes. In our original assays using cavernous sinus plasma, we were using the Simpson-Evans test, which depends upon antrum formation and interstitial cell repair in the hypophysectomized female rat. We can get both antrum formation and interstitial cell repair.

DR. MELAMPY: You also got ventral prostate response, didn't you, with large quantities of plasma?

DR. CLEGG: No. We have never been able to get ventral prostate response.

DR. PARLOW: I think Dr. Clegg is suggesting, in the case of the castrated sheep, that there is some dictum which states that there must be higher serum LH activity than in intact animals.

In this connection, I would like to point out first that we have found in the serum of castrated mice no LH activity at all with the OAAD method even though we readily find LH activity in the serum of castrated rats (Endocrinology 74:102, 1964). Also, no LH activity was found in serum of ovariectomized guinea pigs.

I should also like to point out that there have been studies on urinary gonadotropins in sheep after castration and apparently castration did increase excretion of urinary gonadotropins, but the level of excretion was very low indeed.

It is quite possible that in the case of the sheep after gonadectomy there is a differential increase of FSH and not of LH release.

This is an area of profound species differences. For example, in the case of the rat pituitary following gonadectomy, there is an approximate tenfold increase of FSH concentration and virtually equal increase in LH concentration. On the other hand, in the case of the castrated gilt, according to a study on which I collaborated with Dr. Melampy and Dr. Anderson (Endocrinology 75:385, 1964) there was a relatively small increase in pituitary FSH and LH concentration after ovariectomy. I don't think one can test a bioassay method against what one expects to find on the basis of a particular physiologic manipulation.

I might add, also, at this point, that I personally take a dim view of the bioassay of LH activity in serum by the OAAD method when the source of serum is a heterologous species. In our experience, whereas injection of rat or rabbit or guinea pig serum intravenously into rats is a relatively benign procedure and has no detrimental effect on the health of the assay animal, on the other hand had injection of serum of the human and the heifer and probably serum of other species which I have not tried personally, does have a severely deleterious effect on the health of the assay animals. It may not be a permanent effect, but it is clear on a transient basis that the animal is not well. I believe that this works havoc with the ovarian ascorbic acid depletion response.

DR. NALBANDOV: I think that is a very good point, Dr. Parlow, although I think precautions can be taken such as, for instance, adding known quantities of an LH standard to plasma and trying to recover this by the OAAD test which, I think, to some extent safeguards against what I think you are pointing out. And I think if this procedure is used and the health and well-being of the rats is closely watched, it is not a question of health because they are going to get killed anyway, that the objection that you raised can be circumvented.

The reason I am saying this, I think we ought to explore this much further than we have and I would not want your remark to go down as an indication that we should not be encouraged to continue with this work.

DR. PARLOW: Possibly not, with caution.

DR. KENNEY: Does Dr. Clegg consider the wave-length of light that is used to be important?

DR. CLEGG: We have not specifically studied wave-lengths of light.

The picture has been reviewed by Hammond and, although wave-length does appear to be important in some species, for example, the bird, there is no data to indicate any specific wave-length insofar as sheep are concerned so long as one is within the visible spectrum.

DR. ZIMBELMAN: I would like to ask Dr. Parlow whether there are any species exceptions to the increased FSH secretion after gonadectomy.

DR. PARLOW: If by secretion, you mean release of FSH into the blood, I am not yet certain. There does seem to be species differences with regard to the magnitude of increase of FSH concentration of the pituitary following gonadectomy. As I indicated in our study with Melampy and Anderson (cited above), the increase in pituitary FSH concentration after ovariectomy is relatively small in the gilt, whereas it is very large in the rat. With regard to all the species we have examined following gonadectomy, there has been an increase in FSH plasma concentration, or an increase in urinary output. (We have not found FSH nor LH in the urine of the castrated rat).

DR. NALBANDOV: Anybody else? If not, Dr. Lamond has indicated that he will be kind enough to address a few words to the group. Dr. Hansel will present him. Dr. Hansel.

DR. HANSEL: I don't know exactly why I have been asked to introduce Dr. Lamond. However, I believe many of you may not know much about him. He is a man whom I had never met until yesterday, as you remember, when we happened to cite some of his work and found, by accident, that he was in the audience.

I have, and I think most of us, have had occasion to pay very close attention to Dr. Lamond's work for a long while. He has done excellent early work on assay methods, some of the results of which are perhaps applicable to some of the things we have just been talking about.

More recently he has been interested in ovarian-pituitary relationships, originally in mice. And he has either been graduated or been demoted then successively to sheep or cattle, depending upon how you look at it. And more recently he has been involved in some very interesting estrous cycle synchronization work. He was trained mostly in Australia. His veterinary work was in Australia. He has also worked in New Zealand, and received his Ph. D. from working with Dr. Emmons in Australia.

DR. LAMOND: Thank you, Dr. Hansel. The reason I am giving this small discussion, I guess, is that a number of facts that have been brought to light in the last day or so bear on work that we have been doing in Australia. And it seemed to me that some of the slides that I have with me of some of our work that has already been published and some that is not published would be of interest. The main thing I will stress is the multitude of factors that influence the synchronization procedures.

May we have the first slide? This is some early work that has been published. The main point about this is to indicate the relationship between dose of progesterone, 10 mg. per day, 20 mg. every two days, 20 per day, 40 every two days. The interaction between dose and frequency of progesterone and the time of day of the last injection on the interval from the final progesterone injection to the onset of estrus. Now, the main points about this, I guess, are that first of all there seems to be a fairly close relationship between an optimal progesterone treatment and fertility. There, with 10 mg. per day, four out of seven ewes became pregnant. And, as you go up the board, you find there are certain groups that had no pregnancies at all. Other factors there that show up are that with a lower dose of progesterone the interval is shorter than with higher doses. The important factor, I guess, just for this discussion, is to emphasize what we consider to be one key factor, and that is that the time of day of the final injection of progestrone is important. And you notice here that the variability in this particular group, 20 mg. every two days, when the injection is given in the afternoon is much greater than in some of the other groups. Also, we did laparotomies on these ewes and we find that ovulation under these circumstances, even though there is a lot of variability in various treatments, appeared to occur at the right time, towards the end of estrus.

Next slide. We made use of this response of the period from the final injection of progesterone to onset of estrus, and used this as a quantitative response. We examined the periods of onset of estrus at different times of the year, and this is really the effective dose, the amount of progesterone at different times of the year that would give a period of approximately 80 hours; a constant period of 80 hours. This is calculated exactly the same way as you would an ED in a normal bioassay. The important point seems to be that in February, which is the beginning of our breeding season, the ED is 40 mg. per day; April and May, which is the middle of the breeding season it is 80 mg.; but in July when our sheep are still cycling quite heavily the effective dose, the amount of progesterone that gives an interval of about 80 hours is only 7 mg. Here is a seasonal change in the effect of progesterone in this response. The same general pattern was observed when injections were made every two days, but, of course, you need a bit more progesterone.

Next slide please. Now this is an experiment which is a demonstration of the importance of or the influence of nutritive status on ovarian function. This is going to become our major work or one of our major fields of study in the beef animal. Our problem, of course, is to know what nutritive status is, but we don't go into that one. The important feature of this slide is that Group I, the restricted group, were a group of ewes which were held at about 15 to 20 percent below their normal body weights for the period of the experiment and some time before; Group II, the maintained group, were fed ad libitum; and Group III, the semi-restricted group, were on normal ration prior to the treatment with progesterone and then went on to a restricted ration. Taking the Groups I and III, the key factor seems to be that the period from the final injection of progesterone to the onset of estrus is influenced by nutritive status. In the progesterone alone group you see it is around about the 80 mark; the dose of progesterone in general is 20 mg. per day. Compare that with the dose response relationship in the maintained group. In other words, there appears to be a change in the effect of progesterone associated with this nutritional status. And, when we give PMS at the time of the last injection of progesterone, we find that these relationships differ. In other words, what seems to have happened is that in the animal that is maintained below its normal body weight in a restricted nutritive state, the response of the effect of progesterone is enhanced. And this is overcome by PMS.

The next experiment was brought about because a good deal of work had been done in Australia on the ram effect. This goes back twenty or thirty years, to observations that the rams do influence early season activity and also the activity at the end of the breeding season. Well, in my innocence when I was doing some of this work on progesterone-PMS interrelationships, sometimes the teaser rams would go into the ewes before the experiment, and in this particular instance it just so happened that the final injections of progesterone occurred on a Saturday and so we decided to put the rams in on the Friday afternoon because of the labor convenience, and so forth, and we got this curious estrous story that developed. The progesterone treatments were 10 or 20 mg. given either daily or every two days. We gave PMS two days before or at the same time as the last progesterone injection, and we gave different doses of PMS. Now, remember this, that the last progesterone injection was due on a Saturday, day 0, and that date was the 27th of May. On the Friday, the 26th of May, we put the rams with the ewes. And you will notice this situation, the relationship between the numbers of ewes that showed estrus and the progesterone treatments they received up to that point. Ten mg. every day, three ewes were served within 24 hours of introduction of the rams. In other words, a break through. The rams caused a break through in the progesterone suppressive action. None of these ewes has shown estrus during the treatment. From ovulation studies it was obvious that no ewes had ovulated during the treatment up to this point. With 10 mg. every two days, we got quite a large break through. Because that amount of progesterone is not nearly as effective as a suppressive agent as the daily injections. So here we get quite a large break through. With 20 mg. every two days we also got quite a marked break through, but with 20 mg. per day there was no break through. Now, that, I think, is an important observation because it indicates that the ram stimulus, a neural stimulus, can actually break through the suppressive action of progesterone under certain circumstances. We have looked at this in some detail in numerous ways and it seems to be quite a clear-cut characteristic. There is a clear-cut dose, frequency of injection of progesterone relationship with this ram effect: the more progesterone, the greater suppressive action and the more difficult it is to break through.

The next one, please. This was a fairly large experiment, a seasonal experiment, in which we had various groups of ewes in yards for over 12 months of the year. Now this group here, the top one, was a group of 50 ewes. Rams were with this group for the entire time except that we had a battery of about 12 vasectomized rams, and there would be three with these ewes for a week and then we would put another three in, and so forth. Now that is the estrous story--the percentage of ewes in the group that showed estrus in any 18-day period, in this case the periods that ended on these particular dates. And, of course, you get the normal sort of seasonal pattern. Here we have anestrus, and here the breeding season and beyond this anestrus again. The important point here, an observation that has been made in Australia now a number of times, is that where you have rams running with ewes all of the time there are some ewes that do not show estrus every cycle, and that is why this difference occurs between 80 percent and 100 percent. When we examined the ovulation story in the same ewes, and we did laparotomies on half the ewes every six weeks to determine the ovulation picture. We found as we might expect that about 100 percent of these ewes were ovulating throughout the breeding season and that at both ends of the breeding season there were silent ovulations. This is a fairly clear-cut observation that has been made in Australia a few times now. Silent ovulations are quite a normal occurrence under these circumstances before and at the end of the breeding season.

The next group is a group of 32 ewes which were receiving progesterone treatments. These were getting progesterone for two weeks. In this instance the rams were introduced the day after the final injection of progesterone and were left in for a week. For the remaining five or six weeks in the series, and in between each series, the rams were kept not less than six feet away from these ewes. In other words, they were in the same area; the ewes could see them; they could smell them but they were not in direct contact. And yet we still got this very marked ram effect. You will notice that a fairly reasonable level of ewes showed estrus during this week after progesterone treatments. The thing to do is to compare that line with the next line, which represents the data from a group of another 32 ewes which received identical progesterone treatments but had the rams with them all of the time. So the only difference between the two treatments or the two groups of ewes is that in one instance the rams were introduced the day after the final injection of progesterone and were left there a week, and in the other instance vasectomized rams were with the group all the time. Notice the major differences, and these are highly significant, at the beginning and at the end of the breeding season. Here we have got clear-cut suppression of ovarian activity at the end of the breeding season by progesterone as well as a ram effect. In actual fact, of course, this is a fairly quantitative thing. But the main point I want to get over is that we have a clear-cut interaction between the ram, progesterone, and time of year, on estrus.

This experiment is part of a series of experiments in which we were doing more detailed studies on progesterone-PMS interactions. This thin line with the crosses represents a group of ewes that received 10 mg. of progesterone per day. The heavy line with the crosses represents ewes that received 14 mg. every two days. We picked these two treatments because they happen to be equivalent doses so far as the response we are interested in is concerned, that is the period from the final injection to onset of estrus. They give the same estrous pattern. So that is the 10 mg. per day and 14 mg. every two days. The closed lines are where we gave PMS on the day of the final injection and the broken lines are where we gave PMS the day after the final injection, so we have a large factorial experiment involved here. The point is that the numbers of ovulations are clearly influenced by the frequency of injections of progesterone. Where the progesterone was given daily we have got few ovulations with this high dose of PMS, but where the progesterone injections were given every two days, we got a marked increase in the numbers of ovulations. Now this has been confirmed a couple of times at different times during the breeding season using different doses of progesterone. In follicle numbers, there are no major differences. The duration of estrus, however, was related to the treatment with PMS. We get the normal kind of estrous pattern with the two lower levels of PMS when the PMS is given at the time of the last progesterone. But, when we give the PMS the day after the last injection, we get this marked increase associated with the dose of PMS in the length of estrus. Look at this, 88 hours duration. And here is an obvious source of trouble, of course, in synchronization work. What this amounts to is that the optimal combinations of progesterone and PMS are very, very fine indeed. And you only have to be a little bit off one way or the other and you are going to get abnormalities obviously in estrogen and progesterone relationships.

The next slide, please. Well, this is some of the cattle work that probably has been published by now. We expressed the corpora lutea in 48 Hereford heifers, gave 20 mg. of progesterone of six days, then we gave two dose levels of PMS, and then two or three days after that we gave differing levels of HCG. Now, all that indicates is that by the use of progesterone, PMS and HCG, it is possible again to find an optimal treatment. Here we have five out of eight of these heifers in one group went in calf to the insemination. In the other groups you have got none and one. That is all that indicates. I am fairly convinced that the same general principle holds in the cow as in the ewe, that with the use of these various hormones you can get fairly sensible pictures, once you know what combinations of levels to use. The rest of all this work was done in an attempt to find a technique that could be used in the field. Well, of course, this kind of treatment, where you are giving PMS and HCG under fairly controlled conditions, is not satisfactory in the field, and these other experiments indicate the kind of field experiments we did. It doesn't matter a great deal about the treatments. We gave progesterone, sometimes alone and sometimes with PMS, sometimes with HCG. This is just a small sample of many hundreds of cattle and sheep. You see this sort of a picture, in some groups we got 9 out of 20 in calf to the synchronized estrus, 13 out of 24 here, then we go down to 0 out of 9, and so forth. The point being that in all the work I did in cattle I could not find any sensible pattern once we got away from this kind of a treatment. While we were using progesterone, PMS, HCG, we got fairly sensible stories. Once we got onto progesterone alone, and progesterone plus PMS, or progesterone plus HCG, we just did not seem to be able to get a sensible story out of it. And I think this is probably due to the fact that there are so many factors that can influence the response to these hormones.

The next slide please. Now, this is an experiment, just to bring us up to date on what we are now doing. And this is another factor, I think, of vital importance in our work. We did this with just a few heifers in a preliminary experiment, five on the low plane, and six on the high plane of nutrition. They were 12 to 14 months old when we started treating them last October. The rations were standard rations. Now the low plane ones were the ones whose body weights were roughly constant. Both groups were treated with progesterone in oil every second day for four injections followed by six daily injections of thirty mgs. plus 1500 I.U. or 2000 I.U. of PMS. This is just one of the many standard kind of progesterone-PMS treatments you can give. In the first run we found that the low plane heifers had no large follicles and three ovulations out of five heifers. The higher plane ones had nine large follicles and seven ovulations out of six heifers. A standard sort of a business. Heifers same age, different plant of nutrition, more or better ovarian function. We repeated this a month or two later and we find that the animals are getting a little bit older, although the low plane heifers are not getting any bigger, but there is this seasonal story coming in, as usual. The response of the heifers has improved a little bit. One large follicle and five ovulations in the low plane group, and five large follicles and fifteen ovulations in the high plane group with this treatment. Now the next time we did this we just simply gave the same general progesterone-PMS treatments, but we took them off feed for a period of about 96 hours prior to the final injection. That is, prior to the last day when we gave their final injection of progesterone and their PMS--a 96-hour fast at this critical time. Now that, in fact, isn't such a long time because both groups only lost about 30 pounds each in weight. But look what it did to the ovulations in this high plane group. Here we have got the adapted animals, the animals adapted to a low plane of nutrition, and they didn't seem to be bothered by this starvation period. The rapidly growing animals on a high plane of nutrition were very severely affected by this sort of a thing. We are obviously getting into this in more detail. What this indicates is that even a fast of a few days can influence the kinds of responses we are interested in.

The next slide please. Well, now the final two slides are to impress us if they can, with the diurnal aspects of pituitary-ovarian function. We have been pretty much impressed by these and we have not made much progress, of course, in the sheep and the cow, but we have made some progress in the mouse. This is a table that really describes an FSH assay that we use and have used for a year or two. What I want to impress here from these data is the diurnal picture.

Now this is a situation where we used weaned mice at 18-20 days of age and we hypophysectomize on the next day. We can do fairly large numbers with a rapid technique and we can inject chorionic gonadotrophin, HCG, at varying periods after these hypophysectomies and we find curious diurnal responses. But what we have done, to make a long story short, we have just simply used the old standard HCG-FSH augmentation assay to study diurnal differences. We would simply give 4 I. U. of HCG five hours approximately after hypophysectomy and we compare the morning hypophysectomy response with an afternoon hypophysectomy response. All of these are contemporaneous comparisons, the a. m. and p. m. ones, fairly large numbers, you can see that the fiducial limits are pretty small. Now, this is a very precise assay. It is similar to the one that Igarashi and McCann have just published. We use the hypophysectomized animal whereas they use the normal animal. And I think we probably eliminate a lot of the variability that way. But the important thing is this, on this basis, PMS contains no FSH because those dose levels, 1 I. U. PMS which is the ED in this assay, we get the same result morning and afternoon, and that is the amount that will work on its own without HCG. So there is clearly no augmentation of HCG by PMS in this assay. This is the N. I. H. standard human-pituitary gonadotrophin; 4.3 micrograms is the ED in the morning, down to 2 micrograms in the afternoon and these differences are significant. The Armour pig FSH, this is a two or three year old standard; 4.5 micrograms in the morning, 2.4 micrograms in the afternoon, again, a decrease in the ED. The N. I. H. sheep FSH; 5 micrograms in the morning, 1.98 in the afternoon. It is the same general picture, a two-fold change in ED. Then we look at Armour LH, the horse LH. This stuff contains quite a lot of FSH on this assay but notice that in the afternoon it goes the other way; 12.9 micrograms in the morning, 43 in the afternoon. And we take this fraction down here, one of Ken Ferguson's fractions, an ox pituitary fraction, which is not a very potent one, shows an ED of 12 milligrams in the. morning and 46 micrograms in the afternoon. Now this obviously contains quite a lot of other odds and ends, but what I want to emphasize is that the time of hypophysectomy has clearly influenced the kind of results we got. I am satisfied this diurnal factor or factors can influence a lot of other experiments that are acute experiments studying the diurnal aspects of pituitary-ovarian functions.

The final slide, indicates the results of an experiment along these general lines. This is the standard McClure experiment. He found that when you starve mice for 48 hours starting about day 2 or 3 after the plug was observed that there was quite a large drop in the percentage of mice that were pregnant. Here we have the proportion of mice that are normally pregnant without starvation. Here is the starvation effect. Whether you start this 48-hour starvation at 6 a. m. in the morning on day 3 or 6 p. m. on day 3, that is what happens. So we went back a bit and found that a 36-hour starvation was a fairly critical one. We started it at 6 p. m. on day 2 and 6 p. m. on day 3, 6 a. m. on day 3 and 6 a. m. on day 4. The point is this, that there is clearly an effect, diurnal component, if you like, in the number of mice that lost their embryos in this particular study. We find that if you start the 36-hour fast in the a. m. on either of these two days the losses of embryos are insignificant. If you start the 36-hour fast in the afternoon on either of these two days, the losses in embryos are significant. All I want to suggest is that we need to be very, very careful in what seems to be very simple experiments that, if there is any possibility of an endogenous diurnal rhythm influencing the experimental result, it makes interpretation so much more difficult. We must be aware of this all the time in this sort of work. I think these are the main points I had to offer.

DR. SIERK: We will entertain questions.

DR. CLEGG: I would like to direct a question to Dr. Lamond. I think your results on diurnal variation following hypophysectomy are not only startling but a bit uncomfortable as far as I am concerned. And I would like to ask Dr. Parlow if he is still here, have you made any similar studies on diurnal variation on OAAD?

DR. PARLOW: No, I haven't. You are probably familiar with the paper which appeared in Endocrinology (74:493, 1964) which suggested that there are diurnal variations in the base line level of ovarian ascorbic acid in the pseudo-pregnant rat ovary. The paper suggested that this may be important for the assay method. However, we don't agree. The timing of the assay is such that you must start it at about 9 or 10 o'clock every morning or on the morning you are running the assay in order to complete it by the end of the day, so there is really no problem.

DR. CLEGG: Well, I was thinking, let us say you were running standards in the morning and unknowns in the afternoon.

DR. PARLOW: If you use a randomized block design? In any case we inject all the animals over a period of 2 hours.

DR. CLEGG: All right.

DR. SIERK: Are there other questions for Dr. Lamond?

Well, let me say, first, that we are very happy, Dr. Lamond, for your contribution here and also for the other contributions that have come from the floor. I think these have added considerably to our conference.

Are there others here who have, shall we say, a short statement they would like to make reporting some of their work? We would be very happy to have them. Dr. Hulet.

DR. HULET: I have heard in the aisles and corridors some comment on the numbers of individual ewes which could be mated to a given ram at a particular synchronized estrus. I have heard estimates of ten to twelve ewes. We have had some interest in this, too, in some of our mating behaviour work at Dubois. This past year we wanted to determine a little more of the range of the possibility in the potential mating capacity of our rams in this synchronized situation. We had six rather large breeding groups of ewes. They varied from about 59-62 head of ewes per group. These were in three breeds, the Targhee, Columbia, and Rambouillet. Three groups within each breed. We selected at random one of these breeding groups which we called the control in which there was no synchronization. We had two other groups in which we synchronized estrus. We placed these ewes with vasectomized rams and when they started coming in heat we sorted them in a cutting chute at 8-hour intervals. Those ewes which were in heat and which were marked by a ewe marking harness, were placed with the fertile rams. They were then left with these rams for a period of eight hours. Those which were marked by the fertile rams were then taken out of breeding. Those which were not were left with this particular ram for another eight hours and then were removed. At 8-hour intervals all of those ewes which had come in heat were placed with the fertile rams and then the majority of them were removed after a period of eight hours.

I have here a histogram which shows the distribution of lambing from matings under this situation compared to the controls. This is the distribution of lambings from these matings. These here at the top are those from the control ewes which were not synchronized. Those on the bottom show the matings at the first synchronized estrus, the second and the third. There is variation introduced here by difference in length of gestation due to breed which probably increases the variation beyond what might have occurred had there been only one breed involved.

To summarize, the percentage of ewes which lambed to the first synchronized estrus was 66 percent when approximately 60 head of ewes were mated to one ram, the ewes being synchronized. The fertility in the control group was only 45 percent. This was due, however, to the fact there was one very lowly fertile ram in this group. I would like to mention, too, there were two rams which were used in the synchronized ewes which we call "no work" rams which we have redefined as sexually inhibited rams. These rams at semen testing time show no interest in either estrual or non-estrual ewes. We find that after introducing them into breeding some of them eventually will breed. Breeding is delayed as long as one week to ten days in some of these rams, whereas the majority will begin breeding within a 24- to 48-hour period. We had two of these rams in this group. One of these rams failed to mate for the first 48 hours and then began breeding. Also the fertility in one of these rams was down. So I think this 66 percent is a rather conservative figure. So I think perhaps some of us underestimate the ability of rams to breed a large number of ewes successfully. Thank you.

DR. HANSEL: May I ask one question of Dr. Hulet?

Was that a pre-selected ram?

DR. HULET: No. None of these rams were pre-selected, except that they were selected by Dr. Ereanbrack in the breeding program. The selection was based on their breeding index, their genetic value, rather than on any mating behaviour characteristics.

DR. TERRILL: Do you think the results would have been different if you had not cut the ewes in--if you had just left the rams with all the ewes all of the time?

DR. HULET: Probably not a great deal of difference, although I think this does give a little advantage to the ram. In our papers which we published on mating behaviour there is a very strong tendency for the rams to favor those ewes which were just coming into estrus compared to those which had been in estrus for some time. So there tends to be a natural selection of new ewes so a ram tends to distribute himself rather than confine his attention to a particular ewe.

DR. SIERK: Dr. Rankin of Squibb.

DR. RANKIN: I have debated whether I should mention what I am about to discuss. I talked to Jim Wiltbank and he thought I ought to bring it out.

There is a growing body of literature most of you people are not familiar with which is called the Federal Register. In June of this year proposed regulations were written on the disposal of animals on which experimental drugs had been used.

It is always a surprise to me the number of people who are in academic work and completely unaware of the trends that are taking place in the control of these animals. We have been blessed by the ability to use large numbers of animals and to salvage them for food.

This salvagability is going to come to a screaming halt in sixty days from the 19th. You have the opportunity to write objections or comments regarding these regulations. I would not want to attempt to read them except to state that they are in the June 19th Federal Register. If anyone would like a copy, let me know and I will undertake to supply you with one. But it greatly restricts the distribution of experimental drugs and the use of animals on which experimental drugs have been used, that is on the salvage of these animals on which experimental drugs have been used. I would like to take this opportunity to call this to your attention. Thank you.

DR. GIER: Dr. Gier, Kansas State University.

I have some information I think would fit in with a lot of the things that have been going on here. I have been working to a considerable extent with dogs for the last several years. They have considerable advantages for studying estrous cycles, primarily because they do not have so many of them. One is not crowded up on top of the other, so we have a good opportunity to tell what factors are actually being involved.

Three years ago I started on some work with anti-estruals in bitches. I started with the Upjohn product, Prodox, a substituted progesterone, given orally in daily feedings. These were given in individual feedings so we knew exactly how much each animal was getting. On the low level, 2.5 mg. per kilogram of body weight was fed for a year's time so that we covered at least one and usually two estrual cycles. These animals recovered from the effect of the Prodox on an average of 80 days. That is, they ovulated on an average of 80 days after the end of a full year's treatment. Of five animals on this particular test, all ovulated, four of them had palpable fetuses at 20-25 days. One of them carried through to parturition and produced one pup. On the seven animals that were on a higher level, 3.5 to 5 mg. per kilogram body weight, the minimal recovery was 85 days and the maximum was about 250 days, as I remember; it was several months. Of this group all but one produced litters. She ovulated but there was no evidence of conception. The average litter was 6.2. This is about average for my laboratory. That is, there was full recovery after this heavier dose of Prodox.

I interpret these results, along with some histological work on the uterus and ovary, to mean that the pituitary recovered from the effects of this material more rapidly than the uterus did. The uterus was still under some of the effects of this material even after three months lapse of time, but the pituitary was ready to go. But on the heavy doses, the uterus was completely recovered before the pituitary was recovered and functioning.

I have carried this work on more recently with Provera, which is MAP. This was injectable, single dose. The recovery ranged anywhere from six to thirty months from a single injection, as much as thirty months before there was any recovery. Now, the recovery from this has been very irregular. A few of the bitches have conceived and produced litters. But in most of the cases the progestational effect on the uterus lasted longer than the repressive effect on the pituitary. They come in heat. They may even ovulate. But the uterus is in no condition to maintain the pregnancy. Thank you.

DR. HANSEL: What breed of dogs are these?

DR. GIER: They were primarily Terriers and Cocker Spaniels. There were two Beagles in the total group. I don't remember which group the Beagles were on; one on each level, I believe.

ANONYMOUS: Did you see any cystic glandular hyperplasia in those uteri?

DR. GIER: Oh, yes, lots of it. Great big ones. Anywhere from individual uterine gland cysts to extreme expansion of the uterus. There were seven cases out of fifteen in which there were very definite palpable pseudopregnancy conditions, swelling, enlargements on the uterus, just like pregnancies of anywhere from 20 to 40 days. This was all due to an actual secretion of the uterine glands in both cases. We had two cases in which bacterial contamination was recovered from many of these swellings. But this stuff stimulates the uterine glands exceedingly. I did not get a similar type of stimulation from the Prodox. The lower levels of MAP do not give the progestational effect on the uterus that the higher levels do. I had some that went as low as 50 mg. per dog and it kept the bitch out of heat for at least eight months. The uterine effects were nowhere near the uterine effects of the higher doses.

My original doses were 125 to 200 mg., which I think is excesssive for the bitch. I think 50 mg. is a much more reasonable dose.

DR. SIERK: We will entertain an additional question or comment that anyone has.

DR. DZIUK: The topics so far have been devoted primarily to the domestic animal and the laboratory animals.

I would like to interject an idea here that is not new but has not been mentioned. That is the possibility of using these compounds in pest control. I am thinking here, for example, of starlings and even such things as deer, raccoon, opossum, skunk, and so forth. I think there is a possibility, particularly in bird populations, that some of these compounds could be useful in controlling populations much more effectively than poisoning, shooting, and that sort of thing.

DR. SIERK: Dr. Cole has agreed to some discussion if we desire on this part. Anyone have a comment that he wishes to make?

I think you summarized it very well, Dr. Cole.

Well, gentlemen, this concludes this conference on Estrous Cycle Control in Domestic Animals. I am sure that it does not conclude the efforts in this direction. At least I hope that it does not.

I wish to publicly thank the people who have participated in the formal presentations, the section chairmen, and their colleagues. Obviously, without their contributions, such a conference as this could not be held.

We sincerely appreciate the attendance on the part of you people who have made some effort to be here. We are very happy, of course, to see the interest and the attendance on the part of industry representatives. We think this is most healthy.

I have one other recognition I would like to make, and this is the very fine facilities that have been provided here at the University of Nebraska. I want to, again, publicly give recognition to Dr. Zimmerman for his efforts in all that he has done to make this what, on the face of it, appears to have been a successful conference.

AFTERNOON SESSION 1 p. m.

DR. SIERK: Several have asked Dr. Gerrits for more information on the material he gave yesterday, so we are going to take a few minutes here at the outset for Dr. Gerrits to present a little supplementary information.

DR. GERRITS: As Dr. Sierk mentioned a number of workers have asked questions regarding the work that we have been doing with a non-steroid in controlling the estrous cycle of swine. I will present a little more specific information on the study with the non-steroid that I made brief reference to yesterday in Dr. Hansel's section.

At the outset I should say that Paget et al in December of 1961 reported the effect of this compound on ovarian function in the rat. Since that time four or five other papers have appeared in the literature and reported the effect of the compound on reproduction in women, horses, and mice.

Projected here is the chemical structure of the compound that we fed to the gilts. Namely, it is 1-α-methylallylthiocarbamoyl-2-methylthiocarbamoyl hydrazine. The compound is produced and patented by an English drug firm and is known as I.C.I. 33,828. It is handled by Ayerst Laboratories of New York here in the States.

The gilts were fed on an individual basis. The level of the compound that they received daily, as indicated in this chart, ranged from .48 to 1.47 mg. per kilogram of body weight. Two animals at levels lower than .8 mg. came into estrus while on treatment. Also you can see that 22 of the 26 gilts showed estrus on either the 5th, 6th, or 7th day after cessation of treatment.

Ovulation at the estrus following treatment was normal. In this respect it was a pleasure to work with this compound in that we did not encounter the incidence of cysts that we have experienced when swine are treated with the orally active progestins.

The conception rate of this group of 26 gilts was 73%. This is based on cleaved ova recovered at 3 days or embryo recovered at more advanced stages of gestation.

Although this study includes only a few animals, the data show that this compound is effective in controlling the estrous cycle of swine. On the basis of this preliminary study it is tempting to suggest that this compound may offer us a much needed and useful research tool and perhaps may provide the impetus necessary to develop a sound program of artificial insemination in swine.

DR. GIER: Who handles this material?

DR. GERRITS: Ayerst Laboratories in New York.

DR. HANSEL: May I ask just one question? I am not certain I am thinking of the right compound, but isn't this the one that prevents implantation as well?

DR. GERRITS: Harper's work with rats does suggest an effect on embryo survival. I believe Dr. Harrington who is in the back of the room has worked with rats on this problem. Dr. Harrington, would you care to make a comment on that?

DR. HARRINGTON: It has not prevented implantation but has brought about mortality subsequent to implantation. I believe Harper also points this out.

DR. HANSEL: And you have looked so far only at fertilization rate, not embryo survival.

DR. GERRITS: No. We have recovered embryos at 16 and 25 days post breeding and our conception rate and embryo survival appear to be normal. Of course, as you realize we have only had a few animals on this and thus cannot draw any real conclusions regarding embryo survival at this time.

It does seem that length of time on treatment as well as total dose of the compound may influence intake. At present we have some other trials under way which we hope will answer some of the many questions that remain regarding the effective use of this compound.

DR. SIERK: Thank you, Dr. Gerrits.

We will proceed now with the summary of the conference. Dr. Cole, of California.

DR. COLE: Thank you, Dr. Sierk, and gentlemen.

COMMENTS AND CONCLUSIONS CONCERNING STUDIES ON ESTROUS CONTROL IN DOMESTIC ANIMALS

H. H. Cole
University of California, Davis

Let me allay at once any apprehension you may have that I am about to recapitulate all that has been said during the past two days. Redundancy at this time would merely accentuate your current soporific state. Thus I shall take advantage of the prerogative of seniority to modify the title of my talk to "Comments and conclusions concerning studies on estrous control in domestic animals."

In reviewing the papers submitted for this conference one is impressed with several facts:

1. Significant progress has been made in the pharmacological control of estrous phenomena in domestic animals. Many of the studies, however, would have been more valuable had the effects of the pharmacological agents, endocrine and synthetic, been studied in greater depth. For example, the only factors considered in several studies included merely the presence or absence of estrus, the time of estrus, and finally, whether mating of treated animals resulted in normal pregnancies. The inclusion of further groups of animals sacrificed at various phases of the experimental period to study the morphology of the reproductive organs, the enzymatic concentrations in the tissues, and/or the hormonal levels in the blood, urine, and the pertinent endocrine glands would, hopefully, have led to a more detailed knowledge of what was happening and given a clearer insight into the causes of success or failure of the treatment. I should add, hurriedly, that no one has been more guilty than I in this respect.

It might be helpful to diverge for a moment to enumerate the steps in research I consider essential before a natural or synthetic compound is ready for practical application in controlling reproductive phenomena. First, a screening test indicates that a compound may have usefulness for a given purpose. If the compound has been synthesized or purified from natural sources by a pharmaceutical house, this company has an interest in seeing that its biological properties are screened. Secondly, more detailed studies of the biological properties of the compound are made. This step would also involve studies on the optimal levels and the time interval of administration of the compound. Funds for sophisticated studies on the influence which hormones or synthetic compounds with hormonal properties have upon physiological processes are available from a number of Federal agencies. It is this second step which many tend to avoid. In my opinion faster progress could be made in extending the frontier of knowledge concerning reproductive processes if these studies in depth were made, either before or concurrently with extensive trials in large numbers of animals under field conditions. There is, of course, a pitfall in my suggestion; namely, that concentration on more basic studies may divert your attention completely from the practical problems which need your attention. The final step involves the field tests to which I have referred. Many times these field trials would be more rewarding if they were more refined as concerns procedures. This lack of refinement is, in most cases, due simply to the lack of adequate funds and this is a problem to which the USDA might direct its attention. State funds are being exhausted merely to take care of the exploding student population. Formerly more funds were available in the Agricultural Experiment Stations for applied than for basic research but this trend has been reversed.

2. It would seem that further conventional studies with MAP and CAP could hardly be justified. I'm reminded of the song from Oklahoma, "Everything's up-to-date in Kansas City, We've gone about as far as we can go." Certainly we have gone far enough to know that feeding of these compounds leads to considerable synchronization in cattle and to remarkably good synchronization in sheep. If one can bring 80% of a group of cows into estrus within a 3-day period with 40-60% pregnant at first service this becomes an entirely practical expedient in a range operation. The results in cycling ewes are even more striking.

Of particular interest to me is the high degree of success obtained by Brunner et al. (1) in the induction and synchronization of estrus in anestrous ewes by the use of MAP combined with PMS. This finding, of course, does need additional confirmation with ewes of several of the important breeds and under various environmental conditions.

The studies of Hansel, of Casida and his co-workers, and of Dzuik point up the special problems encountered with the use of synthetic progestogens in swine.

3. I have only commendation for the studies reported concerning the factors influencing persistence of the corpus luteum, for the studies on the role of the hypothalamus in reproduction and for studies on the role of social and environmental factors in controlling or modifying reproductive activity. The elusiveness of the mechanism whereby the uterus exerts its effect upon the ovary is intriguing. These interesting fields can be profitably exploited in greater depths with assurance

that much of practical importance will be forthcoming. No doubt some of the differences in results obtained in synchronizing estrus are due to differences in environmental factors.

4. Reference has been made to the possibility of using antigonadotropins in estrous control. Our own studies provide little encouragement for success by this means in the near future. High titers of anti-HCG are easily attainable and we have shown that this antibody is effective against pituitary LH. However, the cross reactivity with LH is very poor and therefore the amount of antiserum needed to inhibit the LH response is prohibitively high. On the other hand, pituitary FSH is a relatively poor antigen and consequently we have not been able to develop sufficiently high antibody titres to make practical use of anti-FSH in controlling estrus.

5. A disappointing feature of the studies reported is the paucity of information on the steroid and gonadotropic hormone levels following treatment with MAP or CAP. Methods of studying steroid hormone levels are readily available. The Parlow ovarian ascorbic acid depletion method provides a means whereby pituitary LH levels may be determined in the cavernous sinus blood plasma in the ewe (Dierschke and Clegg, unpublished). Unfortunately, an equally sensitive test for pituitary FSH is not available.

As a general conclusion the advances made in estrous control in cycling and in anestrous domestic animals are most gratifying. My plea, however, is that we study reproductive phenomena in greater depth and likewise study the effect of pharmacological agents in greater depth. If we know secretory rates of the various hormones controlling reproductive activity and if we know more accurately the enzymatic reactions associated with specific reproductive activities, we will, in turn be able to evaluate more accurately the normalcy of the response to pharmacological agents--both normal occurring hormones and synthetic chemical compounds.

We should exploit more fully new techniques which have been developed:

1. Yesterday, Dr. Anderson spoke of studies on hypophysectomy in domestic animals. These studies are laudable but we need to proceed with caution in concluding that hypophysectomy is complete. Recently, Lostroh et al (2) has shown that the ovaries of hypophysectomized rats can be further depressed by the administration of anti-LH indicating that there is still residual tissue secreting LH. Reports on the gonadotropic activity of median eminence tissue provide further evidence of the difficulty of the clear-cut removal of all tissue secreting gonadotropins.

2. Chemical methods are now available which make it possible to study secretory levels of steroid hormones involved in reproduction. We have been slow in studying these levels in the several domestic species under various reproductive states.

3. The immunochemical approach provides another means of purifying gonadotropic preparations. It has been possible to obtain LH preparations of high purity but no one ever claims to have obtained pure FSH by chemical procedures alone. Li and his associates have treated purified FSH preparations with anti-LH and shown that further purification can be obtained by this means. His results are in essential conformity with those which Snook and I have obtained concerning the biological properties of FSH. It appears that FSH alone has no effect on the female but will cause growth of the seminiferous tubules of the male with no influence on the male accessories.

4. Immunological techniques will be useful no doubt, in studying levels of gonadotropins in tissues when sufficiently pure gonadotropins are available as antigens.

5. Tapping of cavernous sinus blood draining the pituitary in sheep as developed by McFarland, Clegg, and Ganong (3) represents a real advance in methods of studying gonadotropic secretory roles.

6. The Parlow ovarian ascorbic acid depletion test (Parlow, 4) represents a more sensitive means of detecting LH concentrations. Even this method, however, is not sufficiently sensitive to accurately assay LH in peripheral blood.

7. As Nalbandov has indicated, we now have a means of studying the control of the secretion of gonadotropins by the hypothalamus. This provides us with a means of determining the mode of influence of environment on reproductive phenomena.

What, in essence, I am trying to say is that I think too many of our studies are of an empirical nature. We need to know more about basic reproductive physiology in order to more intelligently approach problems such as estrus control. The very considerable success which has been obtained by empirical methods should not blind us to the limitations of this method of approach. As a former department chairman, I am also apprehensive about the tremendous cost in the long run of this method of approach.

Literature Cited

(1) Brunner, M. A., Hansel, W., and Hogue, D. E.
1964. Use of 6-Methyl-17-Acetoxyprogesterone and Pregnant Mare Serum to Induce and Synchronize Estrus in Ewes. Jour. Anim. Sci. 23: 32.

(2) Lostroh, A. J., Johnson, R., and Gordon, C. W., Jr.
1963. Effect of Ovine Gonadotropins and Antiserum to Interstitial Cell Stimulating Hormones on

the Testes of the Hypophysectomized Rat. Acta Endocrinologica. 44: 536.

(3) McFarland, L. Z., Clegg, M. T., and Ganong, W. F.
1960. Concentration of ACTH in Cavernous Sinus and Peripheral Blood Collected from Unanesthetized Sheep. Proc. Soc. Exp. Biol. Med. 103: 538.

(4) Parlow, A. F.
1961. In Albert A. (Ed.), Human Pituitary Gonadotropins. Charles C. Thomas, Springfield, Ill., p. 300.

(5) Snook, R. B., and Cole, H. H.
1964. Endogenous Gonadotropic Activity in Mare Serum Subsequent to Chronic Treatment with Gonadotropin. Endocrinology 74: 52.